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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Environmental Health Criteria Document on Methyl Bromide for WHO

FROM: Vivian A. Williams, M.S. 6/2/92
Biologist
Toxicology Section II
Toxicology Branch I
Health Effects Division (H7509C)

TO: Gary Burin, Ph.D.
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THRU: Karl Baetcke, Ph.D. 6/4/92
Chief
Toxicology Branch I
Health Effects Division (H7509C)

Action Requested: Review the submitted draft document for methyl bromide by placing specific emphasis on the correct citation of the toxicology literature, determining if any significant work has been omitted, and commenting on the general structure and content of the document.

Conclusion: The methyl bromide document adequately characterizes the available information concerning the physical, chemical and analytical properties of methyl bromide as well as the human\consumer\occupational and environmental exposure aspects and the adverse health and environmental effects. The most noteworthy modification to the toxicology section is to delete all references to the "draft" 1990 NTP 2- year mouse inhalation data and replace it with the recently released final version of the data which is dated March 1992, (Technical Report Series Number 385). Most of the additional comments are basically informational since they mainly deal with the current regulatory status of methyl bromide.

The Office of Toxic Substances prepared a document called a Chemical Hazard Identification Profile (CHIP) on methyl bromide that is dated September 28, 1984 and revised February 20, 1985. That document contained a summary of readily available health, environmental effects, and exposure data. The methyl bromide document drafted by WHO is constructed in a similar format as the

CHIP document and they both share a number of common references. The WHO draft document is, of course, more current in its literature citations. Comments regarding the WHO draft document are as follow:

The primary route of exposure for humans is through inhalation, although adverse dermal exposure scenarios have been described and several of the cited references have discussed the occupational exposures and poisoning incidents involving the general population.

The section on neurotoxicity in animals is somewhat detailed, however, the same type of detail is not reflected in the neurotoxicity section on humans. It is well understood that in human incidences, the exact exposure levels are difficult to determine, however, the document fails to suggest whether the exposures were high or low and no length of exposure time was provided.

As stated in the document, there are no available immunotoxicity data in our possession for this chemical.

On Table 8.4.2.1, page 186, the reference to the NTP study should be aligned to the text which describes the dose and effect for the 13 week study. In the next entry on the same table, there is a discussion of the effects wherein "pituitary gland tremour (male)" is cited; this is thought to be a typo.

On page 188, the NTP is cited as a reference, but it is unclear as to which data on the table it is being associated.

For your information, I have attached a copy of the memo (dated June 12, 1991) which lists the toxicity data requirements for the data call-in for methyl bromide. I have also attached a methyl bromide status report (dated 1/17/92) from the California Department of Food and Agriculture Medical Toxicology Branch. This information represents our most recent regulatory activity for this chemical.

ATTACHMENTS

Copley

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

June 12, 1991

MEMORANDUM

SUBJECT: Methyl Bromide, Toxicity data requirements for the new DCI

FROM: Marion Copley, DVM Marion Copley 6/12/91
Section Head, Section 4
Toxicology Branch 2, HED

TO: Lois Rossi
Reregistration Branch
Special Review and Reregistration Division
(FAX 308-8773)

THRU: Karl Baetcke, PhD, Chief Karl Baetcke 6/12/91
Toxicology Branch 2, HED

1. 83- guideline series requirements

SERIES	STUDY TYPE	GAP	TIME FRAME
83-1a	chronic-rat (safety[1])	data gap	Standard time for completion of new study OR six months if company wishes to submit an existing study. Is their responsibility to assure that the study will meet Agency criteria.
83-1b	chronic-dog (safety[1])	data gap	3 years
83-2a	onco-rat (inhal)	data gap	Standard time for completion of new study OR six months if company wishes to submit an existing study. It is their responsibility to assure that the study will meet Agency criteria.
83-2b	onco-mouse (inhal)	data gap	Standard time for completion of new study OR six months if company wishes to submit an existing study. It is their responsibility to assure that it will meet Agency criteria.

[1]dietary safety study high dose must be at least 100X anticipated residues of Methyl Bromide.

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83-3a	**devel-rat**	(inhal)	data gap	Standard time: Although the study is considered acceptable in the 1986 Registration Standard it is brief, the microfiche only includes every other page. Although the study may be adequate, this presentation is inadequate to determine core classification.
		(safety)	reserved	To be based on results of chronic safety and residue studies.
83-3b	**devel-rabbit**	(inhal)	no gap	An acceptable study is present.
		(safety)	reserved	To be based on results of chronic safety and residue studies.
83-4	**repro-rat**	(inhal)	no gap	An acceptable study is present.
		(safety)	reserved	To be based on results of chronic safety and residue studies.

2. **90-day inhalation study on pyrolysis smoke** - required if cured tobacco has residues of .1 ppm or greater.

3. **90-day inhalation-rabbit study** - required in 1986 Registration Standard due to proposed increased sensitivity in the rabbit. Comparison of rat and rabbit teratology studies suggest that the rabbit is not more sensitive than the rat therefore this study is not required.

4. 85-1 **Metabolism-rat** data gap Reserved in the 1986 Registration Standard. Now required since MBr residues are found in certain crops. this requirement may be satisfied by from the open literature.

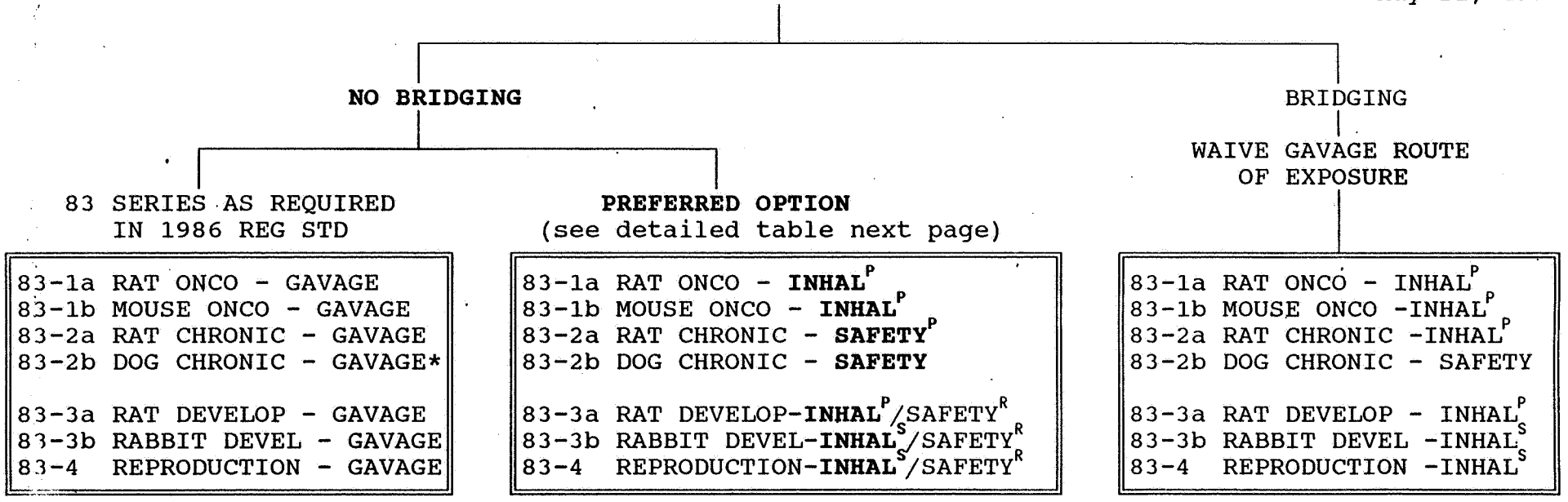
5. **Neurotoxicity** - The acute and subacute battery including Functional Observational Battery, Motor Activity, and Neuropathology, is required since MBr has been associated with in neurotoxicity (1986 registration Standard).

   81-8ss **Acute neurotoxicity-mammal** gap Standard time

   82-5b **90-Day neurotoxicity-mammal** gap Standard time

6. It was agreed during a meeting between OREB, TB1 and W. Burnam that additional exposure data will not be required at this time. However HED has identified exposure concerns and will send a memo regarding the label improvement program and manual needs under separate cover.

METHYL BROMIDE DECISION TREE FOR 83- GUIDELINE SERIES May 21, 1991



* Gavage route no longer required due to excessive toxicity IN THE DOG.
Bridging - implies acceptable study demonstrates that oral and inhalation routes have similar chemical distribution.
Bolded studies - Final HED recommendation
S - acceptable study available.
P - study available, may possibly be determined upgraded to acceptable.
R - Reserved

OTHER GUIDELINE REQUIREMENTS FROM THE REGISTRATION STANDARD

82 SERIES - Inhalation required in standard in rabbit. This is to be reevaluated based outcome and comparison of the rat and mouse NTP chronic/onco bioassays, and rat and rabbit inhalation studies. Subchronic rat requirement will probably be satisfied by either the Netherlands study and/or the NTP chronic studies.

84 SERIES - Studies for chromosomal aberration and other genotoxic effects are currently underway. Gene mutation was listed as satisfied in the 1986 Standard.

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CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

NAME OF ACTIVE INGREDIENT:
METHYL BROMIDE

SB 950 #078, Tolerance # 123

October 20, 1987
Revised May 10, 1989
Revised July 12, 1991
Revised January 17, 1992

I. DATA GAP STATUS

Chronic rat:	Data gap, no study on file
Chronic dog:	Data gap, no study on file
Onco rat:	Data gap, inadequate study, possible adverse effect indicated
Onco mouse:	Data gap, no study on file
Repro rat:	Data gap, inadequate study, possible adverse effect indicated
Terato rat:	No data gap, possible adverse effect
Terato rabbit:	No data gap, possible adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome:	No data gap, no adverse effect indicated
DNA damage:	No data gap, possible adverse effect
Neurotox:	Not required at this time[1]

[1]The brain is a target organ for inhaled methyl bromide; its neurotoxicity is being handled presently under the other sections wherein neurological data have been developed or will be developed.

Note: Toxicology one-liners are attached.

** indicates acceptable study.

Bold face indicates possible adverse effect.

Revised file name: T920117
Revised by: Stephen J. Rinkus, 1/17/92

EPA Reregistration guidance document dated August, 1986 contains EPA findings.

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II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

CHRONIC, RAT

Note: No acceptable study involving chronic-toxicity testing in rats is on file. A chronic rat study of the methylation products and bromine residues resulting from fumigation of rat feed is found in record 095929. EPA had indicated a need for an acceptable rodent chronic-toxicity study using an oral route of exposure (gavage) in its 1986 Re-registration "Guidance" document. CDFA MT is requiring chronic-toxicity testing in rats using an oral route of exposure that does not involve bolus dosing like gavaging. (Rinkus, 7/12/91).

123-127 095929 "Two-Year Oral Chronic Toxicity and Carcinogenicity Study in Rats of Diets Fumigated with Methyl Bromide," (Mitsumori et al., Fd. Chem. Toxic. 28:109-119, 1990). This study used F344 rats (both sexes) to examine the chronic toxicity and carcinogenicity of methylation products and bromine residues resulting from fumigation of rat feed with methyl bromide. After fumigating the feed to attain ~500 ppm total bromine, the feed was exposed to air for 3 weeks; this feed was then pulverized and mixed with untreated feed to achieve dose levels of total bromine of 200 and 80 ppm. Actual organic methyl bromide levels were not determined in this study, except to note that at the end of the 3-weeks airing, the level of organic methyl bromide in the feed containing ~500 ppm total bromine was < 20 ppm. The only effect observed in this study was body weight depression in males fed the diet containing 500 ppm total bromine; the effect was attributed to methylation products generated in the feed since a comparable effect was not seen in rats fed a diet containing 500 ppm KBr. **Supplemetary information. No workheet. (Rinkus, 5/3/91).**

SUBCHRONIC, RAT

123-043 913094 A 90-day subchronic rat study (Danse et al., Tox. Appl. Pharm. 72: 262-271, 1984) indicated a carcinogenic response in forestomach at 50 mg/kg. (Wong, 4-8-85). However, a reanalysis of the histological slides of Danse et al. by a NTP panel concluded that the lesions appeared to be non-neoplastic only (inflammation and hyperplasia) (see letter of 5/9/84 from Dr. Boorman [NTP] to Dr. Vos [National Institute of Public Health, The Netherlands] in front of CDFA document 123-103). (Rinkus, 4/25/89). However, while Hubbs (record 059183 in CDFA document 123-083) also did not find any carcinogenicity in rats treated up to 17 weeks with 50 mg/kg, Boorman et al. (Toxicol. Applied Pharmacol. 86: 131-139, 1986) did observe an early carcinoma in one of 11 rats treated for 25 weeks at 50 mg/kg. (Rinkus, 4/17/90).

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/17/89) notes EPA classification as "Core Minimum". CDFA reviewer (Aldous) presumes this to refer only to the subchronic study data requirement, since the 1986 Registration Standard did not consider the chronic rodent study data gap filled. [Aldous, 1/5/90].

123-109 087805 "Histopathology of Acute Toxic Responses in Selected Tissues from Rats Exposed by Inhalation to Methyl Bromide," (Hurtt et al., Fund. Applied Toxic. 9: 352-365, 1987). Methyl bromide (99.9% pure) was given by inhalation to groups of 10 adult male Fischer 344 rats at 0 (air), 90, 175, 250, and 325 ppm for 6 h/day for 5 days; an additional untreated group received feed quantities identical to those consumed by the rats in the 325

ppm group. After the 5th exposure or in extremis (325 ppm, 4 days), rats were sacrificed and the following sites were examined histologically: nasal cavity, brain, liver, kidney, adrenal glands, testes, and epididymides. Ataxia and diarrhea were observed in the rats exposed to ≥ 250 ppm; tremors and/or convulsions were observed in a few rats exposed to 325 ppm; reddish perineal staining (hemaglobinuria ?) in some rats exposed to ≥ 175 ppm; no clinical effects were cited for rats exposed to 90 ppm. Histological findings were: degeneration of the nasal olfactory epithelium (≥ 175 ppm); degeneration in the cerebellar cortex (≥ 175 ppm; two lesions noted: large to small foci of granule cells, with edematous distension of the cytoplasma; and a diffuse granule cell degeneration without the edematous cytoplasma); degeneration in the cerebral cortex (325 ppm) and the dorsolateral regions of the thalamus (325 ppm); hepatocellular degeneration (325 ppm); lipid accumulation in parenchymal cells of adrenal cortex (≥ 175 ppm); and delayed spermiation (325 ppm). No lesions were noted in the kidneys or the epididymides; the former finding indicates that the presumed hemoglobinuria is not due to a renal lesion. The authors compared these lesions to similar lesions seen in rats exposed to methyl chloride (which presumably was at much greater concentrations, e.g., 3000 ppm). Supplemental information. No worksheet. (Rinkus, 2/28/90).

ACUTE, DOG

123-124 091578 "Acute Oral Toxicity Study in Beagle Dogs with Methyl Bromide," (Naas, D.; Wil Research Laboratories, Inc.; project no. WIL-49006; 10/9/90). Methyl bromide, 100% purity, was administered one time orally (corn-oil solutions in gelatin capsules) to beagle dogs (1/sex/treatment level) at 500, 50, 5, 3, and 1 mg/kg; no negative controls were used. Testing at 5 and 3 mg/kg consisted of using two different concentrations of methyl bromide: high concentration (HC), 158 and 138 mg/ml, respectively; and low concentration (LC), 63 and 64 mg/ml, respectively. Dogs were observed daily for clinical signs for 1-2 weeks postdosing, depending on the treatment level. Both dogs treated at 500 mg/kg exhibited severe signs of toxicity and vomiting and were found dead the next day; necropsy indicated toxicological effects in the stomach, kidneys, adrenal glands, and brain. No other dogs in the study died and no other dogs were necropsied. Severe signs of toxicity and vomiting of reddish material (presumably blood) were seen in the dogs treated at 50 mg/kg. The only other clinical sign seen in the other groups was vomiting, which in some cases contained reddish material. No vomiting was seen during the one week postdosing observation period in two dogs treated at 1 mg/kg or the females treated at 5 (LC) and 3 (HC) mg/kg. Supplemetary data. (Rinkus, 11/2/90).

123-124 091577 This record is a letter from the contract laboratory that conducted the acute oral dog study in record 091578 to Great Lakes Chemical Corp. (member company in the MBIP); it describes the observation of vomiting in two dogs treated once with methyl bromide at 5 mg/kg, using gelatin capsules that contained **microencapsulated** methyl bromide. Supplementary information. No worksheet. (Rinkus, 11/2/90).

CHRONIC, DOG

Note: EPA had indicated a need for an acceptable non-rodent chronic-toxicity study using an oral route of exposure (gavage) in its 1986 Re-registration "Guidance" document. CDFA MT is requiring chronic-toxicity testing

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in dogs using inhalation as the route of exposure. The Sponsors have been asked to pay special attention to evaluating the neurotoxic and ophthalmological effects in this study. (Rinkus, 7/12/91).

048 913193(4110) "Chronic Ingestion by Dogs of Methyl Bromide Fumigated Food." (Albany Medical College, 1960) Methyl bromide fumigated food was fed to beagles, 4/group, daily at 0, 150, 75 or 35 mg/kg/day. **No adverse effect indicated:** Apparent NOEL = 75 mg/kg/day (lethargy, obesity, and one death at high dose). **Unacceptable.** Test article not characterized, no analysis of feed over the 6 to 8-week periods in which a given batch of test article was used, no necropsy/pathology data presented, too few animals (only 4 females at all treatment levels combined). J. Wong, 4-8-85.

ONCOGENICITY, RAT

Note: EPA had indicated a need for an acceptable rat oncogenicity study using an oral route of exposure (gavage) in its 1986 Re-registration "Guidance" document.

084 059184 "Chronic (29-Month) Inhalation Toxicity and Carcinogenicity Study of Methyl Bromide in Rats," (Civo Institutes TNO, The Netherlands; report no. V86.469/221044, 1/87). Methyl Bromide, purity 98.8%, administered by whole body inhalation at concentrations of 0, 3, 30 or 90 ppm to 90 Wistar rats/sex per treatment level, 6 hours/day, 5 days/week for 29 months. Decreased body-weight in the females and decreased survival in both sexes were observed in the high-dose groups. Nonneoplastic effects included: irritation of the epithelium of the nasal cavity (hyperplastic changes) in all treatment groups, decreased brain weight for high-dose females; and increased incidence of thrombi in the heart for both sexes in the high-dose groups. **Possible oncogenic effect noted: glioma in 30 ppm males. NOEL = 3 ppm (glioma).** UNACCEPTABLE, but may be upgraded after submission of: 1) clarifications of the histological analyses of the nasal cavity, mammary gland, thymus, blood-bone marrow, and brain; and 2) individual data for time of death and histo-pathological findings. (Kishiyama, 1/20/89; Rinkus, 3/29/89).

123-109 087806, 087807 IARC Monograph on methyl bromide (Vol. 41, pp. 187-212, 1986). No worksheet. (Rinkus, 3/2/90).

123-109 087798 Computer search of the IRIS data base on methyl bromide (bromo-methane). No worksheet. (Rinkus, 6/4/90).

ONCOGENICITY, MOUSE

Note: EPA had indicated a need for an acceptable mouse oncogenicity study using an oral route of exposure (gavage) in its 1986 Re-registration "Guidance" document. Presently, no study by any route is on file. A mouse inhalation oncogenicity study conducted at Brookhaven National Laboratory as part of the National Toxicology Program has been completed and a draft version of the NTP report was given a peer review by NTP on November 19-20, 1990. (Rinkus, 7/12/91).

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REPRODUCTION, RAT

082 058196 "Two-Generation Reproduction Study Via Inhalation in Albino Rats Using Methyl Bromide," (American Biogenics Corporation, Decatur, IL; laboratory study number 450-1525, 2/19/86). Methyl Bromide (lot and purity not stated) was administered to Sprague Dawley rats of both sexes by whole body inhalation 6 h/day for 5 days/week at the nominal levels of 0, 3, 30 or 90 ppm. Parental animals were exposed for about 40 or 55 days and 90-105 days before their first and second matings, respectively, and were exposed for a total of 132-145 days before they were sacrificed. Premating bodyweights were decreased statistically only in F0 males in the 90 ppm group. Absolute brain weights were decreased in F0 males, F1 males, and F0 females in the 90 ppm groups. In the second mating of the F1 parents, the fertility index decreased from 90.9% in the controls to ≤ 68% in the 30 and 90 ppm groups. **Parental NOEL (tentative) = 3 ppm (reduced fertility).** The progeny from the 30 and 90 ppm groups exhibited statistically reduced bodyweights at weaning in each of the four litters produced by these groups. For the female F2b progeny from the 90 ppm group, the absolute weights of the brain, heart, kidneys, and liver were reduced statistically; weight reductions of a lesser degree also occurred for the kidneys, liver, and testes of the corresponding male progeny. **Progeny NOEL = 3 ppm (decreased pup bodyweight and some organ weights).** This study was considered originally by Kishiyama and Rinkus (3/21/89) as unacceptable, but upgradable, pending submission of: 1) lot number and purity of test article; 2) more details about exposure conditions and monitoring; and 3) microscopic examination of target organs in parents per FIFRA guidelines. Item 1 was satisfied with the submission of Attachment 6 (no record number) in document 123-109, but this study remains **UNACCEPTABLE**, pending resolution of items 2 and 3. (Rinkus, 6/4/90).

094 059912 Protocol to 082 058916. No worksheet; not reviewed. (Kishiyama, 3/21/89).

123-139 111505 This record concerns the analytical measurements of the methyl bromide atmospheres generated in record 058196. These supplementary data have not been reviewed, pending submission of the F1 target organ histology data. (Rinkus, 1/17/92).

123-109 087804 "Evaluation of Spermatogenesis and Sperm Quality in the Rat Following Acute Inhalation Exposure to Methyl Bromide," (Hurtt, M.E. & Working, P.K., Fund. Applied Toxicol. 10: 490-498, 1988). Methyl bromide (99.9% pure) was given by inhalation to adult male Fischer 344 rats at 0 (air) or 200 ppm for 6 h/day for 5 days. Rats from both treatment groups were sacrificed (5 or 10 per group, depending on the day) at the following times: days 1 (first day of exposure), 3, 5, 6, 8, 10, 17, 24, 38, 52, and 73. At day 5, the methyl bromide-treated group weighed ↓10% less than the control group and continued to weigh less till day 52. The methyl bromide group exhibited lower plasma testosterone on days 1, 3, 5, and 6 and and a decrease in nonprotein sulfhydryl in the testis and liver on days 1 and 3. Endpoints that were not affected were: clinical signs; testis weight; testicular and epididymal histology; daily sperm production; cauda epididymal sperm count; sperm morphology; sperm motility; and linear sperm velocity. However, CDFA notes spermatocytes and differentiating spermatogonia were sampled only once each (days 52 and 73, respectively); this could be important for sperm parameters like sperm count, morphololgy, and motility. The authors compared these test results with those seen in rats inhaling 3000+ ppm methyl chloride in a similar acute exposure. Supplemental information. No worksheet. (Rinkus, 2/26/90).

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TERATOGENICITY, RAT

**123-039 026866 "Teratologic Assessment of Butylene Oxide, Styrene Oxide and Methyl Bromide (Rats)" (Battelle, Pacific Northwest Laboratory, contract no. 210-78-0025; NIOSH Technical Report, July 1981). Pure methyl bromide was administered to Wistar rats by whole body inhalation 7 hrs/day on days 1 to 19 of gestation at 0, 20 or 70 ppm. Some groups received pregestational exposure for 5 days/week over three weeks immediately prior to mating. The following combinations of pre- and post-mating treatments were employed:0/0, 0/20, 0/70, 20/0, 20/20, 70/0, and 70/70 ppm pre/post-treatment. Initially reviewed as: no apparent adverse effects indicated; maternal NOEL = 20 ppm (diminished body weight gain in early to mid gestation); apparent developmental NOEL = 20 ppm (treatment-related skeletal and delayed ossification effects); unacceptable, upgrade possible; J. Remsen (Gee), 9-4-85; C. Aldous, 10/20/87. In the second review by Rinkus (4/13/89), it was concluded that the high dose did not obviously affect dam bodyweights; maternal NOEL was revised to: > 70 ppm and developmental NOEL remained 20 ppm. The study was considered unacceptable, but upgradeable upon submission of: evidence that test material was technical grade; evidence that a MTD essentially was tested; and individual data for mothers and fetuses. The study is now considered ACCEPTABLE because: technical grade material typically is of high purity like that used in this study; while 70 ppm probably is less than half of a MTD, this is a moot point since the high-dose did exert an effect (delayed skull ossification); and the review of the individual data to see if the effect is being mediated by maternal toxicity will be done if necessary in the risk assessment phase. (Rinkus, 5/24/91).

NOTE: The memo from EPA to CDFA addressing differences in data gap status for this chemical (dated 2/17/89) notes EPA classification as having been changed from "Core Minimum" to "Core Supplementary" (but upgradeable).

092 059690 Partial duplicate to 039 026866. No worksheet. (Kishiyama, Rinkus, 4/13/89)

039 026867 "Teratogenicity Investigation of Orally Administered Methyl Bromide." (An investigation "conducted by Dutch authorities" translated for EPA by Great Lakes Chemical Company, 6-81) Methyl bromide, no purity given, was administered to rats by gavage on day 5 to 20 of gestation at 0 (peanut oil), 0.5, 5, 25 or 50 mg/kg. **Unacceptable.** Poor translation, incomplete with no data. J. Remsen (Gee), 9-4-85.

NOTE: This study was not available to EPA for review as of 2/17/89.

TERATOGENICITY, RABBIT

039 026865 "Teratologic Assessment of Butylene Oxide, Styrene Oxide and Methyl Bromide - Rabbits." (NIOSH, 9-82) Methyl bromide, 99.5%, was administered by whole body inhalation to New Zealand White rabbits, 7 hrs/day, day 1 to 24 of gestation at 0, 20 or 70 ppm, 24/group. **Unacceptable.** No individual data, 2 doses only with one too high. J. Remsen (Gee), 9-4-85. It should be noted that neurotoxicity and death were observed in the rabbits inhaling 70 ppm methyl bromide in this study. The onset of the neurotoxicity and death occurred concurrently after about 1 week of exposures. Out of a group of 25 does, 3 were dead by gestation day 10, increasing to a total of 9 dead by gestation day 15, when exposures were stopped; all does in this group except one were dead by gestation day 30. (Rinkus, 1/17/92).

NOTE: EPA did not accept this study for regulatory purposes (see EPA Reregistration Guidance document of Aug., 1986, 123-071, p. 9).

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092 059690 Partial duplicate to 039 026865. No worksheet. (Kishiyama, Rinkus, 4/13/89)

104 066800 Protocol (draft). A letter from Hazleton Laboratories dated January 28, 1988 for a rabbit teratology study indicates a final protocol is pending. No worksheet. (Kishiyama, 1/24/89)

123-127 095930 "Methyl Bromide Inhalation Teratology Study in New Zealand White Rabbits," (Breslin et al.; The Toxicology Research Laboratory, Dow Chemical Company; Laboratory Project Study ID number K-000681-033; 6/18/90). Methyl bromide was administered by whole body inhalation 6 h/d on days 7-19 of gestation at concentrations of 0, 20, 40 and 80 ppm to 15-21 pregnant New Zealand White rabbits/treatment level (part I) or 0 and 80 ppm to 15-16 pregnant does/treatment level (part II); does were sacrificed on day 28. Treatment levels were chosen on the basis of a pilot study, which is now on file at CDPR (record 111266). Maternal effects were limited to the 80 ppm groups and consisted of decreased bodyweight gains and clinical signs indicative of neurotoxicity (part I only, 3 does: right-sided head tilt, ataxia, slight lateral recumbency, lethargy). **Maternal NOAEL = 40 ppm (neurotoxicity). Fetal bodyweight was decreased statistically in the 80 ppm group in part II. Fetal effects that appeared to be the results of treatments included: omphalocele (80 ppm group, part I); hemorrhaging with or without hydrops (80 ppm, parts I & II); retroesophageal right subclavian artery (80 ppm group, part I); gall bladder agenesis (80 ppm, parts I & II); and fused sternebrae (80 ppm, part I; no skeletal analysis in part II). When first reviewed (5/3/91), this study was considered **UNACCEPTABLE**, with a developmental NOAEL of 20 ppm (fused sternebrae; omphalocele); and to upgrade the following had been requested: 1) necropsy data of pups/fetuses of 80 ppm does that delivered early or were found dead; 2) the pilot study; and 3) clarification of matters concerning historical control data, umbilical hernia/omphalocele & number bred in part II. These data have now been submitted (records 111265 and 111266) and, as discussed in worksheet W095930.S01, the matters that they address are now considered resolved. **Developmental NOAEL = 40 ppm (omphalocele, hemorrhaging with or without hydrops, retroesophageal right subclavian artery, gall bladder agenesis, fused sternebrae and decreased fetal bodyweight).** This study now is considered **ACCEPTABLE.** (Rinkus, 1/15/92).

123-137 111265 This record contains the following supplementary information to record 095930: individual responses to the matters raised in W095930.833; the protocol to record 095930; raw data regarding animal observations and (or) the gross pathology examination of two 80 ppm does which either delivered early or was found dead; a table identifying the route of administration used in the studies that comprise the historical control database for the conducting laboratory; an updated version of this historical control database; and some text regarding the management of mucoid enteritis in rabbits. Discussion of this record is contained in the worksheet W095930.S01 **Supplementary information. No worksheet.** (Rinkus, 1/16/92).

123-138 111266 "Methyl Bromide Inhalation Teratology Probe Study in New Zealand White Rabbits," (Breslin et al.; The Toxicology Research Laboratory, Dow Chemical Company; Laboratory Project Study ID numbers K-000681-032 & K-000681-032A; 4/2/90). This study was not a teratology study; rather, it was designed only to evaluate maternal toxicity and embryolethality so that the high dose in a standard teratology study (record 095930) could be set; also histological examinations of the brain (parts I & II) and spinal cord (part II) were performed. Methyl bromide was administered by whole-body inhalation 6 h/d on days 7-19 of gestation at concentrations of 0, 10, 30, and 50 ppm to 4-7 pregnant New Zealand White rabbits/treatment level (part I) or 0, 50, 70, and 140 ppm

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to 6-7 pregnant does/treatment level (part II). Does were sacrificed on day 20, with the exception of the 140 ppm group; these does were sacrificed on day 17 (i.e., after 10 exposure days) due to their moribund state. Clear maternal effects were limited to the 140 ppm group and included: decreased bodyweights and bodyweight gains and clinical signs of neurotoxicity (lethargy, labored breathing, ataxia, right-sided head tilt, reduced sensations in the extremities, dilated pupils, lateral recumbency, loss of placing or righting reflex, and rear leg splay). Histological examinations of the brains of all does on test indicated that only the 140 ppm group had pathological lesions (multifocal areas of inflammation of the meninges overlying most regions of the brain and/or bilaterally symmetrical necrosis or spongiosis of the midbrain dorsolateral to the pyramidal tracts). Fetal examinations were limited to counting the number of implantations and resorptions. A reduction in litter size for the 70 ppm group in association with an increase in preimplantation loss was suggested by the data (no evaluation of 140 ppm group was provided). The authors noted that these effects were not observed again in the full study (record 095930). **Supplemental information. No worksheet.** (Rinkus, 1/16/92).

GENE MUTATION

Note: Document 123-109 contains various published reports regarding the mutagenic potential of methyl bromide. In each case, the experimental details for the mutagenicity testing were not reported adequately, which is often observed with reports published in the open literature. Inadequate documentation of methods is viewed by CDFA as a significant reason for officially rejecting a study. However, CDFA also recognizes that these studies collectively indicate that methyl bromide is a direct-acting mutagen. Since this opinion now is endorsed by the Sponsor also (see Attachment 1 in document 123-109), these studies have been considered collectively as satisfying this data requirement, despite their individual shortcomings. (Rinkus, 2/23/90).

103 066722 "Sex-Linked Recessive Lethal Test in Drosophila Melanogaster," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Two separate stocks of wild-type male fruit flies (D. melanogaster; Oregon K) were exposed to air containing either 20 or 70 ppm of test material for 5 h and subsequently were mated to Muller-5 females to produce F1 females, which were mated to produce the F2 progeny in which the frequency of lethal mutations was scored (Muller-5 test). Treatments with test material did not produce any signs of toxicity or affect fertility. An increased frequency of lethals that was observed for the 20 ppm group using one stock of males was not similarly observed in the corresponding group of the second stock of males nor in either stocks treated at the 70 ppm level with test material. UNACCEPTABLE and not upgradable because testing up to a MTD clearly was not achieved and the testing failed in other ways to meet the EPA guidelines for this assay. (Kishiyama, 2/2/89; Rinkus, 4/6/89).

123-109 087801 "Mutagenic Activity of Chemicals Identified in Drinking Water," (Simmon et al., In: Progress in Genetic Toxicology, Scott et al. (Eds.), pp. 249-258, Elsevier/North Holland Biomedical Press, 1977). Methyl bromide (purity not stated) was tested in the Ames test using TA100; testing did not involve the use of any metabolic activation system like S-9. The experimental details were not described adequately. Agar plates containing bacteria were incubated for 21 h at 37°C in 9-liter dessicators that contained methyl bromide concentrations of 0 (air), 0.01, 0.02, 0.05, 0.10, and 0.20 % (i.e.,

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0, 100, 200, 500, 1000, and 2000 ppm). Stirring bars were used as fans to achieve an even distribution of vapors, but the number of plates per dessicator was not stated. A doubling in the spontaneous number of revertants was seen at the lowest concentration tested; and the number of revertants continued to increase with increasing concentration, up to a maximun effect at the 0.1% treatment level. **UNACCEPTABLE**. No worksheet. (Rinkus, 2/23/90).

123-109 087802 "Mutagenicity of Methyl Bromide in a Series of Short-Term Tests," (Kramers et al., Mutation Res. 155: 41-47, 1985). Methyl bromide of 99% purity was tested for genotoxicity in the following assays: a fluctuation test using Klebsiella pneumoniae; the Ames test using Salmonella typhimurium strains TA100 and TA98; the induction of forward mutations at the TK locus and at the HGPRT locus using L5178Y mouse lymphoma cells; the induction of unscheduled DNA synthesis (UDS) using freshly isolated rat liver cells; and the induction of sex-linked recessive lethal mutations using Drosophila melanogaster. The experimental details were not described adequately. Exposures to methyl bromide were accomplished by: exposing the tester organisms to vapors formed in closed containers into which an ethanolic solution had been introduced (fluctuation test, Ames test); adding an ethanolic solution directly to gas-tight bottles ~90% filled with cell media (mouse lymphoma assay, UDS assay); or exposing the tester organisms in a chamber to a continuous flow of methyl bromide-containing atmospheres (Drosophila). Methyl bromide was active in all tests, except the UDS testing. Lowest treatments that exhibited a positive effect were: 1) fluctuation test, 4750 mg/m^3 (1271 ppm; the estimated concentration of methyl bromide in the nutrient broth was 250 µM); 2) TA100, 1900 mg/m^3 (508 ppm) (no mutagenicity seen with TA98); 3) L5178Y cells, ~0.3 µM; and Drosophila, 3 weeks of 6 h/day, 5 day/week using 200 mg/m^3 (52 ppm). UDS testing conducted up to a maximum concentration of 0.3 mM did not detect an effect, but it was not stated whether the HDT was sufficient to cause cytotoxicity. **UNACCEPTABLE**. No worksheet. (Rinkus, 2/23/90).

123-109 087803 Abstract to work discussed in record 087802. No worksheet. (Rinkus, 2/26/90).

123-109 087808 "Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems," (Moriya et al., Mutation Res. 116: 185-216, 1983). Methyl bromide (purity not stated) was tested for mutagenicity using the Salmonella typhimurium strains TA100, TA1535, TA1537, TA1538, and TA98 and the Escherichia coli strain WP2 hcr. Experimental details were not reported adequately. Testing involved placing one bacteria-containing agar plate without its lid upside down in a glass container, injecting gaseous methyl bromide into the container, and incubating for 2 days at 37°C while an electric fan stirred the atmosphere in the container. The lowest test concentration to increase the revertant frequency of TA100 was ~500 mg/m^3 (134 ppm). Other strains listed as showing a positive response were: TA1535 and WP2 hcr. It was stated without data that the mutagenicity of methyl bromide was not greatly affected by the use of a S-9 mix. This study also indicates that chloropicrin, which is often combined with methyl bromide in formulated fumigant products, was mutagenic in WP2 hcr and TA98 in the absence of S-9 and in TA100 in the presence of S-9; the chloropicrin testing involved the standard plate assay. **UNACCEPTABLE**. No worksheet. (Rinkus, 3/2/90).

123-109 087809 "Estimation of Genetic Risks of Alkylating Agents. VI. Exposure of Mice and Bacteria to Methyl Bromide," (Djalahi-Behzad et al., Mutation Res. 84: 1-9, 1981). Methyl bromide (purity not stated) was tested for mutagenicity using Escherichia coli Sd-4, but the experimental details were not reported adequately. Also, adduct formation of methyl bromide with hemoglobin and DNA in test-tube reactions and in mice exposed to methyl bromide by either

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inhalation or by intraperitoneal injection was determined. Inhalation exposure involved the use of a static system in which 9 mice in an 11-liter chamber inhaled an atmosphere for 4 h that initially contained 36 or 17 ppm (CDFA calculation of ppm concentration). Intraperitoneal exposure involved the single injection of a corn-oil solution to give a dose of 417 µg/kg bodyweight. Bacterial mutagenicity was observed at test concentrations of ≥ 4 mM; the LD50 for these test conditions was 6-8 mM. N-7-methylguanine formation was 10 times greater in DNA isolated from the spleen than that measured in the liver (only organs sampled) of mice inhaling the high dose; DNA adduct formation was not assayed for the low inhalation dose or for the intraperitoneal exposure. Protein alkylation was 22 times greater in RBCs than in the liver for mice inhaling the high dose; protein alkylation was also measured at the low inhalation dose and in the intraperitoneal experiment. UNACCEPTABLE. No worksheet. (Rinkus, 3/8/90).

CHROMOSOME EFFECTS

Note: EPA is requiring both bone marrow and sister chromatid exchange tests (see EPA Re-registration Guidance document of Aug., 1986). With the acceptance of record 099090, this data requirement is now considered satisfied. No adverse effect was observed in record 099090, but CDPR MT is aware that 843-type data also were generated in the NTP mouse oncogenicity study, which the Sponsor intends to submit when it becomes available. A draft version of this NTP study indicated that methyl bromide was active in tests for the induction of SCEs and micronuclei in female mice exposed for 2 weeks by inhalation. (Rinkus, 1/17/92).

044 035750 [Previous Record # = 913095-1] "Effect of Methyl Bromide on the Frequency of Sister Chromatid Exchanges (SCE) in Chinese Hamster Ovary (CHO) Cells." (Pasadena Foundation for Medical Research, 1980) Methyl bromide, purity not given, was assayed with Chinese Hamster Ovary cells at 0, 1, 6, 13 or 26 ppm for SCEs. **Possible adverse effect:** dose-related increase in SCEs. **Unacceptable.** Protocol not provided, criteria for scoring SCEs not provided. J. Wong, 4-8-85. [There is no apparent merit in seeking to "upgrade" this study, as EPA is requiring additional studies of this type in any case].

103 066721 "Cytogenetic Analysis of Rat Bone Marrow Cells," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole body inhalation at concentrations of 0 (air), 20 and 70 ppm to Sprague Dawley rats of both sexes. One group of 30 rats/treatment level received only one 7-h exposure and another group of 10 rats/treatment level received 5 consecutive daily exposures of 7 h/day. The former were sampled at 6, 24 and 48 hours posttreatment whereas the latter were sampled 6 hours posttreatment. There was no obvious treatment-related increase in the frequencies of chromosomal aberrations in any of groups receiving test material. NOEL > 70 ppm. UNACCEPTABLE and not upgradeable because the HDT is at least half of a MTD. (Kishiyama, 1/30/89; Rinkus, 4/4/89).

103 066719 "Dominant Lethal Testing in Male Rats," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole body inhalation at concentrations of 0 (air), 20 and 70 ppm to 10 male Sprague Dawley rats/treatment level for 7 h/day for 5 consecutive days. After the fifth exposure, males were housed with pairs of virgin, non-treated females for 7 days, with a different pair of females being used weekly for a total of 10 consecutive weeks. Examination of the ovaries

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and the uterine contents indicated no genotoxic effects or reproductive effects, as can be measured in this assay. NOEL > 70 ppm. UNACCEPTABLE and not upgradeable because the number of males per treatment level was only 10 and the HDT was at least half of a MTD. (Kishiyama, 2/1/89; Rinkus, 4/4/89).

123-136 099090 "Micronucleus Cytogenetic Assay in Mice" (Putman, D.L. & Morris, M.J.; Microbiological Associates, Inc.; study number T9413.122; 5/17/91). Methyl bromide (purity not stated) was tested for the induction of micronuclei in bone-marrow polychromatic erythrocytes of ICR mice of both sexes. Testing involved one-time intraperitoneal injections of 5 mice/sex/dose and sacrificing them 24, 48 or 72 hours later. Doses based on analytical determinations were: 0 (corn oil), 28, 57, and 123 mg/kg; the targeted low, mid and high doses had been 34, 68, and 136 mg/kg, respectively. The selection of the high dose was based on LD50 data that were contained in the report. **No induction of micronuclei was observed whereas the negative control and positive control (triethylenemelamine, 0.25 mg/kg IP) gave appropriate results. This study is considered **ACCEPTABLE**. (Rinkus, 1/14/92).

123-108 085429 Proposed protocol for conducting a micronucleus test in mice, using intraperitoneal injection as the route of exposure. No worksheet. (Rinkus, 4/20/90).

DNA DAMAGE

Note: EPA is requiring an unscheduled DNA synthesis test using rat hepatocytes and a test to determine the effects on germ cells (see EPA Re-registration Guidance document of Aug., 1986).

044 **913095 "In vitro Microbiological Mitotic Recombination Assay of Methyl Bromide Using S. cerevisiae D3." (SRI International, 4-80) Methyl bromide, purity not stated, was assayed for mitotic recombination with Saccharomyces cerevisiae D3 at 0, 0.05, 0.075, 0.1, 0.15, 0.2, 0.3, or 0.4 % w/v. The study was conducted on 4 days, total of 5, 10 or 15 plates per concentration, with and without activation. Increase in number of mitotic recombinants with increasing dose. **Acceptable.** J. Wong, 4-8-85.

103 066718 "Unscheduled DNA Synthesis Assay," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Unscheduled DNA synthesis was measured in human embryonic intestinal cell after exposure to methyl bromide gas in air at concentrations of 5, 10, 20, 30, 40, 50, 60, or 70%. None of the methyl bromide treatments induced any increase in UDS. UNACCEPTABLE but upgradeable upon submission of a more detailed explanation of how the cells were exposed to test material, the number of cultures per treatment level, and cytotoxicty data. (Kishiyama, 1/30/89; Rinkus, 4/6/89).

103 066720 "Sperm Abnormalities Test in Mice," (Inveresk Research International Ltd., Scotland; report no. 1190, 5/30/81). Methyl bromide was administered by whole-body inhalation at concentrations of 0 (air), 20, and 70 ppm to 10 B6C3F1 hybrid male mice per treatment level. Mice were sacrificed 5 weeks later and their sperm were catergorized in terms of the frequencies of abnormally shaped sperm. There was no significant increase in the frequency of abnormally shaped sperm in the mice treated with test material. **NOEL > 70 ppm.** UNACCEPTABLE but may be upgraded upon submission of purity of test material and toxicity data that supports the conclusion that 70 ppm is a reasonable approximation of a MTD. (Kishiyama, 2/2/89; Rinkus, 4/5/89).

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123-109 087799 "Methylated Purines in Human Liver DNA after Probable Dimethylnitrosamine Poisoning," (Herron, D.C. and Shank, R.C., Cancer Res. 40: 3116-3117, 1980). DNA isolated from the liver and kidneys of a single victim of methyl bromide poisoning (no details at all on this poisoning) did not contain any detectable amounts of 7-methylguanine or O^6-methylguanine. Supplemental information. No worksheet. (Rinkus, 2/22/90).

123-109 087800 "Evaluation of Genetic Risks of Alkylating Agents. IV. Quantitative Determination of Alkylated Amino Acids in Haemoglobin as a Measure of the Dose after Treatment of Mice with Methyl Methanesulfonate," (Segerback et al., Mutation Res. 49: 71-82, 1978). Article does not contain any testing results for methyl bromide per se, but it does explain methods and logic for this approach as applied to methyl bromide in record 087809. Supplemental information. No worksheet. (Rinkus, 2/23/90).

123-108 085428 Proposed protocol for measuring DNA single-strand breakage in the DNA of testicular cells isolated from rats exposed by inhalation. No worksheet. (Rinkus, 4/20/90).

NEUROTOXICITY

Note: The brain is clearly a target organ for inhaled methyl bromide (e.g., reviewed in records 059183 & 064742). However, this SB 950 section refers to organophosphate-induced neurotoxicity. The neurotoxicity of inhaled methyl bromide is being handled presently under the other sections wherein neurological data have been developed or will be developed (especially, the dog chronic toxicity study using inhalation as the route of exposure). (Rinkus, 7/12/91).

SUPPLEMENTAL STUDIES

SINGLE AND (OR) REPEATED INHALATION EXPOSURE STUDIES

No Record Number. "The Response Attending Exposure of Laboratory Animals to Vapors of Methyl Bromide" (Irish et al., J. Ind. Hyg. Tox., 22:218-230, 1940). This study involved single exposures of rats and rabbits and repeated exposures for up to 6 months (7.5-8 h/d, 5 d/w) to rats, guinea pigs, rabbits, and rhesus monkeys (rodent and rabbit strains not specified). The study is notable for its findings of neurotoxicity and species differences. The results suggest the following decreasing order of sensitivity to the neurotoxic effects of repeated exposure to methyl bromide-containing atmospheres: rabbits $\geq$ monkeys $>$ guinea pigs $\geq$ rats. Literature reference. (Rinkus, 1/17/92).

4-17 WEEK GAVAGE STUDY, MALE RATS

083 059183 "The Subchronic Effects of Oral Methyl Bromide Administration in the Rat," (Purdue University, Masters Thesis, Ann Frances Hubbs, December, 1986). Methyl bromide was administered by gavage at the nominal concentrations of 0 (peanut oil), 25, and 50 mg/kg/day (5 days/week) to 71, 41, and 71 male Wistar rats, respectively. Rats received treatments until sacrificed at 4, 9, 13, or 17 weeks, with 7-10/group/sacrifice; however, rats in the 25 mg/kg/day

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group were not sacrificed at the two earliest times. Also, some rats in each group stopped receiving treatments after 13 weeks and remained untreated for either 4 or 9 weeks before being sacrificed. Toxicological examination mainly consisted of histological examination of blood, bone marrow and stomach. Food consumption and bodyweights were reduced in both groups receiving methyl bromide. Gross and histological changes were observed in the stomach of most rats receiving methyl bromide and were consistent with damage and inflammation of the squamous epithelium, but no tumorigenesis was indicated. NOEL, MTD < 25 mg/kg/day. Supplemental information. (Kishiyama, 1/24/89; Rinkus, 4/17/89).

Note: record 059183, as a thesis, contains an extensive literature review on methyl bromide. Topics include: poisoning in man by dermal, ocular (?), inhalation, and oral exposure; experimental animal studies; and in vitro studies (mutagenicity, transformation, and cytotoxicity). (Rinkus, 4/25/89).

TOXICOLOGY LITERATURE REVIEW

099 64742 "Toxicology of Methyl Bromide" is some sort of collaborated review, 29 pages long, plus 7 pages of references (with first two pages missing). Authors have affiliations with Toxicology and Pharmacology, Inc., Georgetown University, and Virginia Commonwealth University Medical College of Virginia. The authors' purpose in preparing the review (e.g., as a submission for publication) is not indicated; also, there is no date on the manuscript. Topics include: exposure, pharmacokinetics, human health effects, experimental studies, teratogenic activity, mutagenic activity, carcinogenic activity, and mechanism of action. It was noted that the most recognized effect of methyl bromide was neurotoxicity. No worksheet. Supplemental information. (Rinkus, 4/25/89).

RESIDUE STUDIES, PRE-PLANT AND POST-HARVEST

123-109 087810 "Methyl Bromide Residue Study (Pre-Plant)--Revised Draft," (Bolsa Research Associates; B.R. #10:87, 4/11/88). This record is some sort of partial report on results of measuring organic methyl bromide and inorganic bromide in a variety of crops grown on soil fumigated with methyl bromide. Apparently, no methyl bromide was detected in any crops grown on fumigated soil, while inorganic bromide levels were increased. Supplemental information. Not reviewed; no worksheet. (Rinkus, 4/20/90).

123-109 087811 "Section E: Removal of Residues," (no author or other identification given). This record is some sort of partial report regarding "additional means of reducing methyl bromide residues," presumably after commercial fumigation. Supplemental information. No Worksheet. (Rinkus, 4/20/90).

123-109 087812 "Fumigant Survey: Flour and Flour Products, April-June 1984," (Oregon Department of Agriculture, Laboratory Services Division, Food and Dairy Division; no date). No methyl bromide was detected in 100 flour and bakery mix products. The analytical method that was used had a detection limit of 0.03 ppm. Supplemental information. No worksheet. (Rinkus, 4/20/90).

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123-109 087813 "Determination of Methyl Bromide Residues in Strawberries after Commercial Fumigation," (no author or other identification given). This record is some sort of partial report regarding the loss of organic methyl bromide residues from strawberries fumigated at the Driscoll Strawberries Associates fumigation facility in Watsonville, CA. The analytical method that was used was the headspace gas-chromatography assay of King et al. Data which were not provided were said to indicate an exponential loss in organic residues, such that only 3×10^{-6} ppm would be expected after 8 hours of some sort of unspecified aeration. Supplemental information. No worksheet. (Rinkus, 4/20/90).

MISCELLANEOUS

123-140 112312 This record contains a letter (dated 1/2/92) from Dee Kuhn (the Chemical Manufacturers Association Manager for the MBIP). The letter summarizes a variety of matters discussed at the meeting of October 30, 1991 in Sacramento between the represenatatives of the MBIP and CDPR-MT staff. This record also contains some written text and tables regarding the presentation made on methyl bromide neurotoxicology at the aforementioned meeting by Dr. Michael Gill. **Supplemental information. No worksheet.** (Rinkus, 1/17/92).

NOTE: All studies received by the CDPR Medical Toxicology Branch up to 1/17/92 have been considered in this SUMMARY OF TOXICOLOGY DATA.

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ATTACHMENT I

California Department of Food and Agriculture
Medical Toxicology Branch
SB 950 Response to the Methyl Bromide Industry Panel (MBIP), rebuttal on Methyl Bromide, SB 950 # 78
Rebuttal Dates: 11/20/89 & 11/27/89
CDFA Response Date: 6/4/90

We have reviewed your following submissions: CDFA documents 123-108 (dated 11/20/89) and 123-109 (dated 11/27/89). Our comments on these submissions follow. No worksheets were prepared in the course of reviewing these submissions. Other CDFA findings are contained in the revised SUMMARY OF TOXICOLOGY DATA.

Your additional questions and comments should be addressed through the Registration Branch.

REGARDING WHETHER AN ORAL RAT COMBINED CHRONIC TOXICITY/ONCOGENICITY STUDY IS REQUIRED FOR REGISTRATION OF METHYL BROMIDE AS A FOOD-COMMODITY FUMIGANT

MBIP comments:[1] Any exposure to workers who use methyl bromide as well as to the public will occur primarily by inhalation. The many chronic-type inhalation studies which MBIP has submitted, or has committed to submit as soon as possible, are adequate to satisfy the various SB 950 data requirements for chronic exposure data.

Oral exposure is not a primary route of exposure to methyl bromide. MBIP has submitted records 087811, 087812, and 087813 concerning the loss of organic residues with time after fumigation. Record 087811 discusses without data a residue study in wheat and wheat flour conducted for the Grocery Manufacturers of America (GMA) and presents some data on fumigated corn and rice. In the GMA study, fumigated wheat was said to contain organic methyl bromide 24 h postfumigation; and after milling, nondetectable to a maximum of 39 ppb could be detected. The GMA study was said to have concluded that any trace amounts of residue should be lost before baking. For corn and rice, the supplied data indicate that 9 and 5 ppm were detectable sometime after fumigation, respectively. After the corn was ground three times, the amount detected decreased to 6 ppm and, after baking for 1 h as a slurry, the level was below the detection limit (10 ppb). Likewise, letting the rice "simmer" for 20 min reduced the methyl bromide content to below the detection limit.

Record 087812 is a survey of fumigant residues in flour and flour products conducted by the Oregon Department of Agriculture in 1984. In this study of 100 flour and bakery mix products, no methyl bromide was detected using a method with a stated detection limit of 30 ppb.

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[1] MBIP comments were abstracted from: Dr. Duafala's cover letter (dated 11/27/89), which accompanied CDFA document 123-109; a letter written by Dr. Roberts of the University of Florida (dated 11/29/89) to Dr. Duafala, which also is found in the beginning of CDFA document 123-109; and records 087811, 087812, and 087813 in CDFA document 123-109.

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Record 087813 is a residue study of strawberries conducted in 1984 by Trical and Driscoll Strawberry Associates. Strawberries were fumigated using a 453,070-liter fumigation chamber in Watsonville, CA. Methyl bromide was used at 48 mg/liter, for 3 hours at 18°C. Afterwards, the chamber was aerated from 45 to 180 min and then the strawberries were cooled down to 1°C over the next 1.5 to 2 h. Strawberries were stored at 1°C for up to 96 h or were stored at 1°C for 12 h and then stored at 21°C for up to 84 h. Sampling involved sealing a dry pint of fumigated strawberries in a quart-size freezer bag and placing the freezer bag between slabs of dry ice. Samples were maintained on dry ice until delivered to the Dried Fruit and Tree Nut Processors Association laboratory in Fresno, CA, at which methyl bromide was assayed by the headspace technique of King et al. (ref. 1). The results of this study indicated that strawberries contained: ~9 ppm of methyl bromide after aeration; ~2 ppm after 12 h of storage at 1°C; ~2 ppb after a total of 24 h of storage at 1°C; and no detectable residue after a total of 72 or 96 h of cold storage. The data indicated the following equation for the loss of organic methyl bromide residue from these strawberries during the aeration phase:

$$C = (87.9)\ e^{(-2.16)(t)}$$

where C = methyl bromide residue in ppm, as a function of aeration time
t = hours of aeration
87.9 = methyl bromide residue in ppm immediately after fumigating (C_0)

This equation predicts that only 3×10^{-6} ppm (3 parts per trillion) should be left in the strawberries after 8 h of aeration of the type used in this study.

Therefore, as these postfumigation studies show, any organic methyl bromide residue rapidly volatilizes to nondetectable levels. It is clear from such data that the consumer is not being exposed--and cannot be exposed--to significant levels of methyl bromide from treated food commodities.

In addition, a chronic oral study for oncogenic and nononcogenic effects would provide little useful information beyond that information which already exists. These existing data allow for the establishment of safe levels of exposure for methyl bromide ingestion with regard to toxicity to the GI tract. Limits for methyl bromide concentrations in food which will avoid toxicity to other organs can be derived from inhalation studies and a suitably constructed pharmacokinetic model. Further toxicity testing in the form of a chronic feeding study would be technically very difficult, time-consuming, and expensive, and should have little impact on the risk assessment of methyl bromide, regardless of its outcome. It would be more productive to direct research resources at applying existing inhalation toxicity data on methyl bromide to oral exposure scenarios.

CDFA response: Loss of organic methyl bromide from a food commodity would be expected to consist of two interdependent components, true offgassing of organic methyl bromide and methylation of nucleophilic sites in the food (e.g., in the amino acids and protein). It appears that the offgassing component is being assumed the MBIP to be the dominant component, but determining this to be true would require that other analyses be performed concurrently (e.g., inorganic bromide determination, or use of radiolabelled methyl bromide to measure postfumigation retention in a food). Since the offgassing process would be expected to vary with the postfumigation conditions (e.g., temperature; type of aeration [forced aeration in a fume hood vs. exposure to the open air in a room which is ventilated in a usual fashion]), the entire process also would be expected to vary with the postfumigation conditions.

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Regarding whether the inhalation route accounts for the greater percentage of exposure to methyl bromide, this is inconsequential to deciding this matter about the need to conduct an oral oncogenicity study. Rather, the crux of this matter is whether there is any exposure to methyl bromide when fumigated food commodities are ingested. The MBIP submissions that address this point (records 087811, 087812, 087813) are too inadequate in details and data to allow for a scientific review of them per se. Examples include the following. Regarding the corn and rice data in record 087811, there are no details about the storage conditions or even the method of analysis. In record 087812, there are no details about how a standard curve was constructed, although at least the method of analysis was identified. In record 087813, there are no data about the construction of the standard curve and no mentioning of the controls that would be needed to address the possibility of methyl bromide loss during the storage period on dry ice; also, the amount of data that is provided is minimal and the data which are the basis for the loss-of-residue equation--an equation that has an unexpectedly large decay constant, 2.16, and initial concentration, 87.9 ppm--were not provided (Figure 1 mentioned in the text was left out of the submission).

This question about how much methyl bromide is to be found when fumigated foods reach the marketplace can not be answered by reviewing unsubstantive, partial reports like these. In order to have some sort of meaningful discussion of this matter, CDFA has reviewed some pertinent studies (references 1-6) that are present in the open literature. These published studies indicate the following:

1) In many of these studies, the method of analysis has been some modification of the headspace assay of King et al. (ref. 1);
2) some of the fumigations were performed in the laboratory using small chambers (800, 28, and 29 liters in references 1 through 3, respectively), in comparison to the large fumigation chambers that are used in practice, e.g., the 453,070-liter facility in Watsonville, CA; and
3) not all food commodities are the same in their kinetics for the loss of organic residue; in particular, foods having a high lipid content (e.g., some cereals, nuts, and cacao beans) retain the residue considerably longer (Table 1 in the Appendix).

CDFA would like to comment on these above points.

Headspace Assay of King et al. (Ref. 1).
This assay involves homogenizing a food (grapefruit in the original reporting) with water in an airtight blender. A septum on the top of the blender allows for the withdrawal of headspace, which is then injected into a gas chromatograph to detect methyl bromide. While the simplicity of headspace sampling is attractive, there still remains the problem of how to quantitate the residue, i.e., how does one construct the standard curve. King et al. used two approaches: 1) injecting benzene solutions of methyl bromide into untreated grapefruits before homogenizing; and 2) placing defined methyl bromide atmospheres over the homogenates of untreated fruit. Apparently, the former approach has not been used widely by people using this assay; but it should be noted that it is doubtful that the resulting pocket of methyl bromide solution would diffuse through the fruit, unless a fair amount of time was allowed. The latter approach for constructing the standard curve appears to be used widely, e.g., references 2-4. Once again, time is critical to this method, i.e., one must wait for the partitioning of methyl bromide between the headspace and the liquid phase in the blender to reach its equilibrium. If an equilibrium is not reached, it will lead to an underestimation of the true methyl bromide content in the food commodity that is being assayed.

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How much time that it takes to reach equilibrium is not intuitive, at least not to this CDFA reviewer (Dr. Rinkus). It could be expected to vary with experimental conditions, including the obvious ones (e.g., food's water content and lipid content) and the not-so-obvious ones (e.g., the amount of foaming produced by blending because this creates surfaces and compartments which need to be transversed first before equilibrium can be achieved). Therefore, the time to equilibrium should be determined empirically. Evidence that an equilibrium was reached was not discussed by King et al. in their report; also, it has not been discussed in references 2-4 in which the headspace method has been used. King et al. did show that in the ~20 min that they waited after blending, some 50 to 76% of the methyl bromide spiked into the headspace did partition into the liquid phase. While data of this type still are not proof that an equilibrium has been achieved, such data do provide at least some indication that some partitioning has occurred. Thus, for example, one would have to be cautious about the data reported recently by Stein and Wolfengarger (ref. 4) for fumigated mangos because they observed that only ~5% of the methyl bromide spiked into the headspace partitioned into the liquid phase during the 25 min that they waited after blending. As noted by King et al., each time samples are assayed for residue, it is necessary to construct a standard curve from unfumigated control samples from the same lot of material. This is important because several studies using this technique (refs. 1-3) have shown that the percentage of methyl bromide partitioning into the liquid phase can vary with factors like the following: the type of food commodity, the water and oil contents, age, storage time before fumigation, and size of the fruit.

To summarize CDFA's concern about the headspace technique (ref. 1), this assay has the definite potential to underestimate the methyl bromide content in food commodities. To guard against this, it is essential that the standard curve be constructed properly. These data as well as a detailed explanation of the testing procedure (e.g., a standard operating procedure) need to be submitted in order to allow any residue study to be evaluated.

Laboratory Studies vs. Fumigation Site and Marketplace Studies.
There are vast size differences between the laboratory fumigation chambers used references 1 through 3 and and the actual ones used in practice. Therefore, CDFA considers it important also to have residues studies of food commodities after they have been fumigated commercially and after they have appeared in the marketplace. It is to record 087813's merit that it was a residue study of commercially fumigated strawberries; however, as discussed previously, the reporting of the testing was much too inadequate. Such studies of commercial fumigation will control for variables of the practice--variables that simply can not be addressed in the small scale operations used in the laboratory. These variables include the following: the degree to which methyl bromide gets distributed about a very large chamber containing food commodities; how much is loaded into the chamber; how the commodity is packaged and how these packages are arranged in the chamber; how often the food commodity is fumigated to achieve sterilization; how the chamber is aerated after fumigating; and how the food commodity is stored afterwards before it makes its way to the marketplace.

That there is actually a potential for laboratory studies to underestimate the residue level after commercial fumigation and storage is suggested by the results with apples in reference 5. Assuming that apples are basically like grapefruit, one would expect that 3 weeks after fumigation with methyl bromide the amount of organic residue would be well below parts per trillion (see Table 1). However, Dumas and Bond (ref. 5) reported that apples that had been commercially fumigated 3 weeks beforehand were still offgassing detectable

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levels of methyl bromide. In their study, the apples were placed without blending into a sealed container and the headspace was sampled 24 h later. Since the purpose of their study was not the quantitation of methyl bromide in the fruit, their data only give an underestimate of the actual level present in the fruit. Their data indicates that the apples contained over 1 ppb. The point here is that it seems impossible that fruit that was supposed to have only 10^{-18} ppm levels of residue, according to the laboratory-generated data shown in Table 1, should be releasing detectable quantities of residue into its surroundings.

Foods with High Lipid Content Can Contain Residues at ppb Levels Weeks Later. References 1-3 provide some substantiation to the MBIP's position about the loss of organic residue, in the following sense. Regardless of whether true offgassing vs. methylation is the dominant component accounting for the loss of organic residue, the loss increases with postfumigation time and it can be described mathematically, for a set of postfumigation conditions, as an exponential decay process. Table 1 in the Appendix lists some descriptive parameters for these decay processes (decay constant, half-life value) for various food commodities for which some residue data were found in the open literature. Using these derived equations for the loss of organic residue, extrapolating to later postfumigation times would indicate that sub-parts-per-trillion levels ($<10^{-6}$ ppm) should be reached in 1-2 weeks in the case of some food commodities like fruits. It can be noted that these predicted residue values can not be verified analytically with the headspace method of King et al. (ref. 1) since this method has a detection limit of 1-10 ppb. Whether verification could be accomplished in a laboratory setting using radiolabelled methyl bromide remains to be determined but could be a possibility.

As Table 1 also illustrates, food commodities like some cereals, nuts, and cacao beans--foods having a higher lipid content than fruits--are expected to retain their organic residue significantly longer. In fact, some even have detectable residue on the order of ppb levels weeks later. Therefore, it is inappropriate for the MBIP to generalize broadly to all food commodities from its experience with strawberries, which appear to lose quickly their organic residues. Rather, strawberries represent the very extreme end of a continuum for the loss of organic residues from fumigated food commodities.

Thus, based on the data in Table 1 as well as considering the precedent presented by the finding of apples that were offgassing 3 weeks after fumigation (ref. 5), CDFA can not agree with the MBIP logic that the consumer can not be exposed because the physical-organic properties of methyl bromide dictate it to be so. Rather, CDFA feels it has sufficient reason to conclude that probably some food commodities presently appearing in the marketplace contain organic methyl bromide residues. While the highest levels typically might be only at the ppb level or even sub-ppb level when consumed and foods having high lipid contents typically would be the ones containing these residues, the point here is that there is some residue being ingested by some consumers. Since there is some residue, CDFA must be concerned about the effects of chronic ingestion of these residues, as discussed in the next section.

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Why the Ingestion of Methyl Bromide is of Concern.
The reasons why CDFA is concerned about the effects of chronically ingesting even low levels of methyl bromide include the following:

1) There are compelling structure-activity considerations that indicate that methyl bromide has the potential to induce stomach cancer:
   a) methyl bromide is a direct-acting methylating agent;
   b) consistent with this property, methyl bromide is demonstrably genotoxic in both in vitro and in vivo assays (reviewed in record 087806), including alkylation of DNA (record 087809) and mutagenicity in the mouse lymphoma assay, in the absence of cytotoxicity (record 087802);
   c) as expected, the stomach is a target organ for methyl bromide when the route of exposure is oral (records 913094 and 059183);
   d) several other genotoxic, direct-acting alkylating agents have been shown to induce stomach cancer in rodents; these include: MNU, ENU, MNNG, ENNG, and other direct-acting alkyl nitroso compounds; allyl chloride; epichlorohydrin; 1,3-dichloropropene; 3-chloro-2-methylpropene; bis(2-chloro-1-methylethyl) ether; 3-(chloromethyl)pyridine HCl; ethylene oxide; propylene oxide; β-propiolactone; and styrene oxide (reviewed in ref. 7).

2) Regarding the existing oral and inhalation data base as well as the proposal to do some sort of pharmacokinetic study, two points need to be emphasized:
   a) Inhalation data are not useful for assessing an effect like the induction of stomach cancer because the effect would be route specific. Apparently, this point now is accepted by the MBIP (see the letter by Dr. Roberts [no record number] in the front of CDFA document 123-109).
   b) Oral studies with methyl bromide have used the following durations of exposure: 13 weeks in the study by Danse et al. (record 913094); 4-17 weeks in the study by Hubbs (record 059183); and 13-25 weeks in the study by Boorman et al. (ref. 8). The FIFRA guidelines for a rat oncogenicity study call for a duration of exposure of 104+ weeks. Therefore, for the purposes of SB 950, the existing oral data are entirely inadequate for assessing the longterm effects of ingesting organic residues because of their short durations. Also, of no less importance is the fact that these studies can be considered inadequate due to the limited histopathology that was done on other organs besides the alimentary tract.

3) In the oral study with the longest duration of exposure, the study by Boorman et al. (ref. 8), an early carcinoma was observed in the forestomach of one of the 11 rats in that group. Table 2 in the Appendix compares this finding to the times that the first tumor was reported to have been detected in other oral studies with known stomach carcinogens. The time that the first tumor is detected in a rodent study will vary depending on the experimental design (e.g., serial sacrificing using a previously documented carcinogen vs. lifetime-exposure studies with an untested chemical wherein only rats found dead or dying are examined at early time points). Notwithstanding these considerations, CDFA has to be concerned that it took only 25 weeks of exposure to induce a forestomach tumor, even if the tumor in question was a carcinoma in situ. CDFA recognizes that this tumor induction occurred with a dose that caused obvious irritation to the forestomach epithelium. However, it remains to be shown what the relationship is between tumorigenicity and chronic irritation. This is especially true given that methyl bromide is clearly genotoxic, which distinguishes it from those forestomach carcinogens which are not demonstrably genotoxic, like

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the antioxidant food additive BHT. For example, in Table 2 in the Appendix, MNU given just once by gavage induced tumors without any ulceration; but if this direct-acting alkylating agent had been given daily at the same dose of 40 mg/kg for weeks, as was done with methyl bromide in the study by Boorman et al., one probably would have observed considerable irritation of the stomach epithelium.

4) Regarding any argument along the lines that the organic methyl bromide residues in ingested foods are just too low to be a concern, CDFA would note that, even though it is a circular argument, deciding whether or not to be concerned requires data like the full rat oncogenicity study. These data are used to determine NOELs and, by extension, what residue levels justifiably can be tolerated. Also, as just one example, in the risk assessment for the carcinogenic effects on the stomach induced by ethylene dibromide, water concentrations of parts-per-trillion were associated with a lifetime cancer risk of 1×10^{-6} (ref. 9); therefore, even such low levels could be of a toxicological concern in principle.

In summary, CDFA concludes that there are probably some fumigated foods containing residual organic methyl bromide at the marketplace and therefore are being consumed. Given structure-activity relationships for stomach carcinogens, the inadequacy of the existing data base, and the indication in one oral study that methyl bromide probably was carcinogenic, CDFA concludes that the proper toxicological assessment under SB 950 for the effects of ingestion of methyl bromide requires a lifetime oral rodent study.

Comments about Designing the Lifetime Oral Rodent Study.

1) CDFA is not opposed to conducting the oral exposure by means other than gavage with oil solutions. Alternatives that would need to be tested beforehand for feasibility include: gavaging with water solutions (methyl bromide water solutions up to 1.75 g/100 ml apparently are possible); placing the methyl bromide in the drinking water; and using microencapsulated methyl bromide that is mixed into a rodent chow, as mentioned previously by the MBIP. Of course, in such cases, the analytical data on the dosing materials (stability, content, and homogeneity analyses) that are required as part of the oncogenicity study would take on even greater importance.

2) CDFA is opposed to conducting the oral exposures by feeding rats chow that has been fumigated with methyl bromide if that chow is not handled in such a way as to provide a constant, verifiable dose of methyl bromide per se to the rats. Such a study with fumigated chow would only be useful for studying the chronic effects of ingesting inorganic bromide and the methylated products that are formed from methyl bromide, but this is not a concern that CDFA has at this time.

3) As discussed in this rebuttal under the heading "Chronic, Rat," there is a data gap for chronic-toxicity testing in rats. Therefore, this lifetime oral rodent study should be conducted as a combined chronic toxicity-oncogenicity study in rats.

4) In Table 2 in the Appendix, the only lifetime study whose design would qualify it as a SB 950-acceptable study is the one for epichlorohydrin (ref. 13). Given the hyperplasia observed by Boorman et al. (ref. 8), Danse et al. (record 913094), and Hubbs (record 059183) and the finding of hyperplasia and stomach tumors in rats dosed with epichlorohydrin at 2 or 10 mg/kg (ref. 13), the highest dose level of methyl bromide to be tested probably should not be in excess of 10 mg/kg. Frankly, there are many indications that methyl bromide will be a stomach carcinogen. CDFA would

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urge that this lifetime study be designed to understand its dose response and the relationship between its tumorigenicity and the irritation that it causes.

CDFA action: CDFA is appreciative of the cost and the amount of effort that it takes to conduct a large-scale chronic rodent study like the one in question. CDFA has given careful thought to the arguments made by the MBIP vis-a-vis the potential for methyl bromide to cause cancer, e.g., in the stomach, due to chronic ingestion of residues in fumigated foods. However, for the reasons discussed, CDFA is requiring a combined chronic toxicity/oncogencity study in rats using an oral route of exposure.

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REGARDING THE ASSESSMENT OF THE NEUROTOXIC POTENTIAL OF METHYL BROMIDE

MBIP comments: (No specific comments were made in CDFA documents 123-108 or 123-109.)

CDFA response: The brain is a target organ for inhaled methyl bromide. At this point, CDFA has no information on neurotoxicity when the route is oral, but it can be noted that blood levels of methyl bromide to the brain would be expected to be greater when the route of exposure is by inhalation, i.e., the neurotoxicity could be route specific. As noted previously in CDFA's rebuttal of 5/11/89 (p.3; footnote 5) and in the Summary of Toxicology Data dated 5/10/89, CDFA is concerned about the neurotoxicity potential of methyl bromide. This neurotoxicity is a matter independent of the question of whether methyl bromide is a neurocarcinogen in the rat; also, it is not addressed adequately by simply using brain-weight loss as the criteria (record 059184). CDFA is considering how to evaluate two inhalation situations: 1) longterm exposure to low levels; and 2) intermittent exposure to higher doses.

CDFA is requesting that the following be submitted:

1) the methods and results of the neurological testing done as part of the Dutch-sponsored oncogenicity study in rats (record 059184);
2) the Brookhaven National Laboratory 90-day inhalation study in mice, wherein some neurological testing was performed (this study was mentioned in an untitled MBIP position paper which accompanied the cover letter of Vernon White, dated 7/1/87, in CDFA document 123-082);
3) any neurological data generated in the Brookhaven National Laboratory full study in mice (it is CDFA's understanding that in that study mice inhaling 100 ppm group for less than 6 months had their exposure stopped but 6 months later still developed a neuromuscular condition); and
4) any other pertinent neurological data that are in the open literature (e.g., Anger et al., Scand. J. Work Environ. Health, 7 (Suppl 4): 40-47, 1981; Honma et al., Neurobeh. Toxicol. Teratol., 4: 521-524, 1982).

If the MBIP believes that it should not be required to submit these data, this position should be explained fully.

Depending on the review of the requested data and the outcome of the brain tissue analysis requested to complete the submitted reproduction study (record 058916), CDFA may request additional data regarding neurotoxicity.

CDFA action: Although this type of neurotoxicity does not fall under the SB 950 category of Neurotoxicity, which generally is concerned about delayed organophosphate-induced neuropathies, CDFA intends to treat methyl bromide as a neurotoxin. Neurological testing data are being requested to evaluate this potential, e.g., to determine NOELs. If necessary, acceptance of the chronic studies in which these neurological data were generated will be withheld until these data are supplied.

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CHRONIC, RAT

MBIP comments:[2] The MBIP believes that the Dutch inhalation study in rats is adequate for fulfilling the SB 950 data requirements for the category of Combined Chronic Toxicity/Oncogenicity Testing in rats.

CDFA response: As discussed in the original review of record 059184, this study does not satisfy the data requirements for a chronic toxicity study. The deficiencies include the following:

1. No ophthalmological examinations were performed, as required under the FIFRA guidelines for a combined chronic toxicity/oncogenicity study. These examinations usually are given prior to dosing and at termination of the study. Minimally, all rats in the negative control and the high-dose groups should be examined. Optic nerve atrophy has been described in a worker with intermittent low- and high-dose exposures (ref. 14). Also, given the alkylating ability of methyl bromide, when the route of exposure is inhalation, the possibility of eye effects stemming from direct damage to the corneal epithelium or from glutathione depletion is an important consideration.

2. Hematology studies did not include some measure of effects on platelets or some other measure of effects on the clotting potential of the blood and those studies that were done were conducted too early into the study, i.e., only after 13 weeks and 52 weeks of exposure, as opposed to after 18 and 24 months of exposure and at termination. CDFA would note also the following: a) thrombi were observed as early as at the 2-y sacrifice in the hearts of the 90 ppm males (p. 27 of record 059184); whether this results from the myocardial degeneration that was also present or from some hyper-clotting state remains to be determined; and b) CDFA is asking still for clarification on how the oncogenic potential in the hematopoietic/leukopoietic system was assessed in this study (discussed under the heading "Oncogenicity, Rat."

3. Serum chemistry studies did not include measurements of electrolytes or bilirubin and those studies that were done were conducted too early into the study, i.e., only after 13 weeks and 52 weeks of exposure, as opposed to after 18 and 24 months of exposure and at termination.

4. Urinanalysis studies that were done were conducted too early into the study, i.e., only after 13 weeks and 52 weeks of exposure, as opposed to after 18 and 24 months of exposure and at termination. While no histological lesions were oberved in the kidneys or the urinary bladder in the 90 ppm main groups (pp. 157 and 163, in record 059184, respectively), kidney weights were reduced in both sexes in the 90 ppm groups at the 1-y sacrifice and the 2-y sacrifice (pp. 81-83 and 85/87 in record 059184, respectively). Only the kidney weight reductions at the 1-y sacrifice (N = 10/sex) were statistically significant but the reduced number of kidneys weighed at the 2-y sacrifice (N = 4/sex in the 90 ppm group) would have affected the statistical comparisons.

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[2] The MBIP's comments were abstracted from Dr. Duafala's cover letter (dated 11/27/89), which accompanied CDFA document 123-109.

CDFA recognizes that while some of these measurements are inadequate, they did provide some data. However, for CDFA, the worst deficiency is the lack of ophthalmological studies, especially since the effect could be expected to be route-dependent. Therefore, the MBIP should explore some of the following avenues in the hope of obtaining some data in this regard:

1) conduct the chronic dog study by inhalation;
2) contact those that have performed the chronic rodent studies and arrange to have the eyes examined, if these tissues are still available (note: page 32 in record 059184 indicates that preserved wet specimens and paraffin blocks would be stored till March, 1990; typically such a date is not an exact deadline).

If some way can be worked out to obtain data that address the potential eye effects of chronic exposure to methyl bromide atmospheres, CDFA would be willing to reconsider the acceptability of the chronic portion of record 059184. Otherwise, the data requirement will remain and will need to be satisfied.

CDFA is requiring a combined chronic toxicity/oncogenicity study using oral exposure, as discussed in the previous section. However, this oral study will not address the potential for eye effects caused by exposure to methyl bromide atmospheres. The chronic toxicity portion is being included in the oncogenicity study of the effects of oral exposure to methyl bromide mainly because one would expect any effects on the liver to be dependent on an oral route of exposure; in which case, the serum biochemistry measurements done in the chronic toxicity testing will be valuable in assessing any liver effects.

CDFA action: Record 059184 is not an acceptable chronic-toxicity study in rats. CDFA has suggested some alternatives to compensate for its worst shortcoming, the lack of ophthalmological examinations. However, one way or the other, ophthalmological data for chronic exposure to methyl bromide atmospheres needs to be submitted. In the meantime, this SB 950 data requirement remains unsatisfied, with an unacceptable study (record 059184) on file.

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CHRONIC, DOG

MBIP comments:[2] The MBIP has agreed to conduct a chronic dog study. A protocol has been submitted to EPA for its review.

CDFA response: The route of exposure was not stated. As discussed in the previous section, there is some argument to be made that the route be inhalation.

CDFA action: CDFA will review the completed study when it is submitted. In the meantime, this SB 950 data requirement remains unfilled. An unacceptable study (record 913193) presently is on file, with no adverse effect indicated.

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ONCOGENICITY, RAT

MBIP comments:[3] The Dutch-sponsored inhalation study in rats (record 059184) is adequate for fulfilling the SB 950 data requirements for the category of Combined Chronic Toxicity/Oncogenicity Testing in rats. Regarding the points raised by CDFA about gliomas in that study, the MBIP have the following comments:

1) the Dutch researchers themselves concluded that there was no induction of tumors of any type in their study;
2) since SB 950 defines a chronic health effect as a statistically significant effect, this matter about gliomas should be inconsequential; furthermore, an inconsistently applied regulatory position regarding levels of significance, such as a "moving target" approach in which significance depends on an individual evaluator's opinion (i.e., 0.01 is considered the "cutoff" in one case but 0.05, in another case), can be critized as being capricious in nature (Attachment 4);
3) the finding of 3 gliomas in the 30 ppm group of males can be discounted also because:
   (a) there was no dose response since only the 30 ppm group had these tumors;
   (b) the 90 ppm group was just as much at risk of developing the gliomas as the 30 ppm group as evidenced by the fact that the death rate for the 90 ppm group was not statistically different from the death rates for the controls and the 30 ppm group;
4) the MBIP has asked about historical control data for this study, but such data for a 29-month study are not available.

CDFA response: CDFA would preface its particular responses to the above with the following three comments.

First, record 059184 is a 183-pages summary report; and, given all of the discrepancies in the report, it also should be viewed as being preliminary in nature. The MBIP should appreciate the vast difference in terms of completeness of data between a summary report like record 059184 and the full reporting of such a study, including individual animal data, which essentially is what is required under the FIFRA guidelines; the full report often is larger than the 1,351-page reproduction study (record 058196) that the MBIP has submitted. CDFA realizes that the MBIP was not associated with this Dutch-sponsored project. However, this fact does not alter the need to have the complete data for this study. This need is made all the more necessary in this case because of the many inconsistencies that CDFA has identified in the report (see the 3/29/89 review of record 059184 for details). That such demands for complete data are made by CDFA on other registrants can be verified by the MBIP by asking its members or former members (e.g., Dow) that individually have registered or are registering pesticides in the state of California.

As desribed in detail below, CDFA has multiple questions about the data for several organs. To answer many of these questions, the individual animal data for time of death and pathological (macro- and microscopic) findings need to be submitted. CDFA is insisting that minimally all of the data needed to

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[3] The MBIP's comments were abstracted from Dr. Duafala's cover letter (dated 11/27/89), which accompanied CDFA document 123-109, and from Attachment 4 (no record number), which also is in this document.

answer the questions be submitted. Possibly, this will not entail submitting the entire data base for this study; e.g., the MBIP could contact the Dutch researchers and secure their responses to CDFA's particular questions.

Second, there are compelling reasons for CDFA to be concerned that methyl bromide can induce brain cancer. These reasons include the following:

1) methyl bromide is a direct acting methylating agent;
2) consistent with this property, methyl bromide is demonstrably genotoxic in both in vitro and in vivo assays (reviewed in record 087806), including alkylation of DNA (record 087809);
3) the brain unquestionably is a target organ for inhaled methyl bromide;
4) several other genotoxic alkylating agents have been shown to induce brain cancer in rats; these include: methyl and ethyl methanesulfonates; dimethyl sulfate; propane sultone and methyl aziridine; methyl, ethyl and various other alkyl nitrosoureas; acrylonitrile; and ethylene oxide (refs. 15-19).

Third, in addition to gliomas, CDFA is concerned about other tumor sites in this study. These concerns were described clearly in the CDFA review of record 059184 and in the rebuttal dated 5/11/89. No acknowledgement of these other concerns has been made by the MBIP in its present rebuttal. CDFA presumes that the MBIP has received the 3/29/89 review of record 059814. **If this is not the case, the MBIP should contact the Pesticide Registration Branch immediately so this situation can be rectified.**

CDFA has the following particular responses to the MBIP's rebuttal comments regarding record 059184:

1. As discussed in the original review of record 059184 and reiterated in this rebuttal under the heading "Chronic Rat," this study does not satisfy the data requirements for a chronic toxicity study.

2. The authors of this study concluded that methyl bromide did not induce any tumors, presumably based on statistical comparisons to the respective tumor incidences in the controls. In the case of gliomas, there was no discussion in the report about their rarity in this strain. What bothers CDFA in this case is that the spontaneous incidence of gliomas in these male rats definitely is not just 0/47 (0%). As discussed later in item 4, it is more like 1/100 to 1/67 ($\leq$1.5%). This is a case wherein the biological significance of an observation may not be accompanied by statistical significance. This situation is not uncommon in rodent bioassays with 50 animals/group when the tumor in question has a spontaneous incidence of <1%; e.g., in F344 rats, such tumors sites include: brain, circulatory system, pancreas, stomach, intestines, kidneys, urinary bladder, and ovaries (ref. 20). A similar situation involving gliomas can be found in the recently released NTP cancer bioassay of ethyl bromide (ref. 21).

The "best science" in this situation is to consider all of the pertinent evidence for a neurocarcinogenic effect, including data generated for other SB 950 data requirements. Structure-activity considerations and genotoxicity potential were discussed above. Other important considerations include the following. What is the historical control incidence in this laboratory and (or) in other laboratories with this tumor in this strain? What were the ages of the animals when the tumors were observed? Since neonatal rats have tended to be more sensitive than adult rats to neurocarcinogens (ref. 16), were any tumors induced in the offspring that had in utero exposure in the rat reproduction study (record 058196)? Whether such an approach can be critized as

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being capricious depends on the how scientifically defensible it is for the given circumstances. In this case, CDFA feels that it has sufficient cause to pursue the matter, especially since at this point much of what is being sought is data that CDFA should have been given in the first place.

3. In fact, the authors of this study noted that mortality was higher in both 90 ppm groups in comparison to the respective control groups (pp. 21 & 58 of record 059184). A pairwise comparison between the 30 ppm and 90 ppm male groups was not reported, but given that the survival of the 30 ppm group was comparable to that of the control group, it would appear that such a comparison also would be statistically significant at some of the same time points.

4. CDFA has identified several studies, including some published by the same Dutch laboratory that conducted the methyl bromide inhalation study, that provide some insight about the spontaneous incidence of gliomas in Wistar rats (see Table 2 in the Appendix). On this basis, CDFA feels justified in viewing gliomas in this strain of rat as a tumor typically appearing late in life and having a spontaneous incidence of $\leq$ 1.5% in males and $<$ 0.1% in females. Therefore, the finding of 3 gliomas in 50 males rats (6%) in the 30 ppm group is unexpected. Admittedly, no tumors were reported in the histological data for the 90 ppm group; but mortality was higher in this group, which means that these animals may not have been equally at risk of developing gliomas. Also, there is the question about the histological findings for two 90 ppm males which macroscopically were identified as having a suspect tumor or brain hemorrhage (pp. 100 & 109, respectively, in record 059184).

5. To avoid any ambiguity or misconstructions, communications from scientists to the registrant that the registrant seeks to use in the discussion of data need to be submitted to CDFA in written form. Therefore, if the MBIP has contacted the authors of record 059184 about the availability of historical control data, these written correspondences need to be submitted. Similarly, if the registrant contacts these authors about particulars of their study raised in this rebuttal, these correspondences need to be submitted.

6. An oncogenic effect is not defined in terms of statistical significance in SB 950. This concept, being a "statistically significant adverse effect," only is mentioned with regards to reproductive effects, as discussed in Attachment 4 in CDFA document 123-109. In general, CDFA's guidance with both of these adverse outcomes is to apply the best science in reviewing the data, which, in some cases, can mean focussing on observations that are not statistically significant at the 0.05 level and, in other cases, ignoring effects that are.

After considering the MBIP's comments and rereviewing record 059184, CDFA is not persuaded to change its designation of the induction of gliomas as a possible adverse effect and its classification of the study as unacceptable, mainly because of the incompleteness of the data. **In order to upgrade this study to acceptable, each of the following items needs to be addressed:**

Brain. CDFA requests a full accounting of how the brain was studied and of the circumstances regarding all gliomas observed in this study. The following questions should be answered:

1. How was the histological examination of the brain conducted? How many transversal sections were made; where were their locations? Were these

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methods applied standardly to all brains that were examined or were some brain examinations contingent on macroscopic findings?

2. Hurtt et al. (record 087805) reported that in rats exposed for 6 h/day for only 5 days to 250 ppm methyl bromide, lesions were induced in the granule cell layer of the cerebellar cortex; and similar exposure to 325 ppm also induced lesions in the cerebral cortex and the thalamus. Histological lesions in the brain also were observed independently by Eustis et al. (ref. 26). Given that female brain weights were reduced at the 1-y and 2-y sacrifice in record 059184, the lack of any findings of nonneoplastic lesions in the brains of the 90 ppm rats in this study is surprising. Was the histological analysis conducted in a manner that had these aforementioned effects or neuronal loss (this is suggested by the brain-weight loss) been present, they would have been detected?

3. Exactly how many gliomas were identified in this study, <u>including</u> any that were found in either sex in groups b, c, and d? When reporting these data, please show how many animals <u>actually</u> were examined; e.g., since there is no entry for the brain in the neoplastic lesion data for group c rats (p. 165 of record 059184), were their brains actually examined for such?

4. For each glioma counted in item 3, what was the age of the animal when the tumor was diagnosed? What were the ages of animals of recent control groups at that laboratory that have developed gliomas (e.g., the six control groups mentioned by Kuper et al. [ref. 22] when discussing mammary tumor data)?

5. What were the histological findings for the rats identified in the macroscopic examinations as having <u>brain hemorrhages</u> (90 ppm male in group c; 3 ppm female and 90 ppm female in main groups [pp. 100 & 110 of record 059184, respectively]) or a <u>suspected tumor</u> (90 ppm male in main group [p. 109 of record 059184])?

Based on the answers to these questions and the results of the brain histological analyses of rats exposed <u>in utero</u> in the rat reproduction study (discussed under the heading "Reproduction, Rat"), CDFA will be then in the position of having sufficient data to judge whether the 6% incidence of gliomas in the 30 ppm group of record 059184 warrants being considered a possible adverse effect.

<u>Nasal cavity</u>. In reference 22, historical control data for nasal squamous cell carcinomas in this strain of rats were said to indicate a spontaneous incidence of $\leq$ 3%. Therefore, this tumor is not as uncommon in this strain of rat as CDFA originally had suspected. However, CDFA still is requesting the following:

1. Since all main groups were examined histologically for <u>nonneoplastic</u> lesions in the nasal cavity (pp. 158-159 in record 059184), what were tumor incidences for these main groups (<u>note</u>: neoplastic data for only the 0 and 90 ppm groups were supplied on p. 170 of record 059184)?

2. Exactly how many nasal cavity tumors were identified in this study, <u>including</u> any that were found in either sex in groups b, c, and d?

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3. For each tumor counted in item 2, what was the age of the animal when the tumor was diagnosed? What were the ages of animals of recent control groups at that laboratory that have developed these tumors (e.g., the six control groups mentioned by Kuper et al. [ref. 22] when discussing mammary tumor data)?

4. Were the control groups kept in inhalation chambers? If not, how were they housed and to what were they potentially exposed, by virtue of their sharing the room; e.g., were the controls housed with some treated animals from another ongoing study or were the controls housed in the same room as the inhalation chambers used in this study.

Bone marrow. Methyl bromide induces chromosomal damage in the bone marrows of rats and mice exposed by inhalation (discussed in record 087806). Therefore, CDFA is concerned about the possibility that the hematopoietic/leukopoietic system could be a target organ. The Methods section (pp. 19-20 of record 059184) indicates that bone-marrow spreads were made on the main groups. What were the results of the bone-marrow spreads for the main groups? In general, what is the evidence that leukemias were not induced in this study; e.g., are there any data for blood differentials made on smears done at sacrifice?

Thymus. Eustis et al. (ref. 26) identified the thymus as a target organ for subchronic exposure to methyl bromide, based on the atrophy that occurred in both rats and mice of both sexes. Therefore, CDFA wants the matter about the missing thymus weights for those rats sacrificed at day 734 (satellite group c) addressed. If indeed thymus weights were not recorded, was it by oversight or because no tissue was available in one or more groups? CDFA notes that there were nonneoplastic data for the thymus of rats sacrificed at day 734 (p. 148 of record 059184); these data indicated that involution (atrophy) was noted in 1/6 and 4/7 for the 0 and 90 ppm males and in 1/6 and 1/6 for the 0 and 90 ppm females, respectively. However, these denominators are supposed to be ~10 (e.g., at the top of p. 148 of record 059184, the entry of "sublingual salivary glands" lists 9-10 rats as being examined in the 0 and 90 ppm groups of males and females); therefore, possibly the missing thymuses were those that had atrophied so much that no tissue could be collected. In general, was there evidence that the thymus was affected by methyl bromide in this study, e.g., by increasing the incidence of atrophy and (or) by decreasing the time to appearance of atrophy?

Regarding thymomas, regardless of whatever this laboratory means by this term (i.e., epithelial tumors, with or without lymphoid involvement vs. a lymphoma originating in the thymus), their incidence in untreated female controls is apparently on the order of 1/19 (ref. 22). In another study from that same laboratory (ref. 23), 2 thymomas were seen in $\leq$ 50 untreated females (the actual number of thymuses examined was not stated). These control incidences indicate that the finding of 4/40 in the 90 ppm female group in record 059184 is not unexpected. Therefore, CDFA drops its request for information on thymomas in this study.

Mammary gland. CDFA also is no longer interested in fibroadenoma in the mammary gland of the females of the low- and mid-dose groups, about which CDFA requested data in its previous rebuttal, because CDFA feels it can assume that only those females in these groups with macroscopically detectable lesions were studied microscopically. This was the stated method in references 22 and 23, published studies from this same laboratory. Hence, the data in record

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059184 can be assumed not to indicate incidences of 96% (26/27) and 91% (32/35) for this tumor type in these groups.

Before closing this section, it should be noted clearly that, aside from the oncogenicity portion of this study, CDFA is also requesting the neurological testing portion of this study, for reasons discussed previously in this rebuttal under the heading "Regarding the Assessment of the Neurotoxic Potential of Methyl Bromide."

CDFA action: For the reasons discussed above, record 059184 remains an unacceptable study. Therefore, this SB 950 data requirement remains unfilled, with a possible adverse effect (gliomas) indicated. This study will only be upgraded to acceptable upon submission of the requested data.

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ONCOGENICITY, MOUSE

MBIP comments:[2] The MBIP will submit the inhalation study in mice conducted at Brookhaven Laboratory as part of the National Toxicology Program. The projected peer review by NTP scientists of this report is March, 1990; therefore, sometime afterwards the report will be made available to CDFA.

CDFA response: Aside from the oncogenicity portion of this study, CDFA is also requesting that the neurological testing portion of this study, for reasons discussed previously in this rebuttal under the heading "Regarding the Assessment of the Neurotoxic Potential of Methyl Bromide."

CDFA action: CDFA will review the report in question when it is submitted. In the meantime, this SB 950 data requirement remains unfilled, with no studies on file.

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REPRODUCTION, RAT

MBIP comments:[4] The two-generation rat reproduction study (record 058196) is adequate for fulfilling the SB 950 data requirements for a reproduction study. Regarding the points raised by CDFA about that study, the MBIP have the following comments.

1) Microfilm of the raw data will be submitted to CDFA as soon as possible.
2) Page G-2, which was in the originally submitted document, is again submitted to show the method of methyl bromide analysis (attachment 5 [no record number] in CDFA document 123-109).
3) The certificates of analysis for 3 cylinders of "Meth-O-Gas" (methyl bromide) sent from Great Lakes to Toxigenics are provided; these show that the purity was 99.9% (attachment 6 [no record number] in CDFA document 123-109).
4) The purpose of a reproduction study is to determine the effects of a chemical on the following: gonadal function, estrus cycle, mating behavior, conception, parturition, lactation, weaning, and the growth and development of the offspring. Record 058196 accomplished this purpose. The brain histopathology data requested by CDFA are not necessary or needed since brain effects were covered in the Dutch-sponsored rat inhalation study (record 059184). Therefore, the fact that the brain was not investigated as a target organ should not impact on whether this study is considered adequate for the fulfilling of this SB 950 data requirement.

CDFA response: CDFA has the following responses to the above comments.

1. CDFA did not request any raw data for this particular study in its original review of this study (dated 3/21/89), in its rebuttal of 5/11/89, or in the August, 1989 meeting with the MBIP representatives. CDFA is not requesting any raw data from this study now. Possibly, this matter is being confused with the CDFA request for data from the rat inhalation teratogenicity study conducted by Battelle Pacific Northwest Laboratory for NIOSH (record 059690).

2. Attachment 6, indicating a purity of 100% for the test material used in record 058196, resolves the matter about purity. CDFA understood from the August, 1989 meeting that such pure material was typical of the technical grade material sold as methyl bromide; therefore, the matter of whether the test material was technical grade, which is what is required for testing under the FIFRA guidelines, will be considered resolved. However, Attachment 5, which CDFA did review when first submitted, does not explain some experimental conditions that CDFA views as important to understanding how the methyl bromide atmospheres were generated and mainitained and how their monitoring was performed. **Therefore, these following items still need to be provided:**

a) total airflow rate through the chambers (to allow calculation of T_{90}); also, were the airflow rates constant;
b) defining the start and finish of the 6-h exposure period with respect to the times that the atmosphere generator was turned on and off; and
c) defining the physical locations in the chambers for atmosphere sampling, with respect to the breathing zone of the rats and the inlet port by which the test material entered the chamber.

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[4] The MBIP's comments were abstracted from Dr. Duafala's cover letter (dated 11/27/89), which accompanied CDFA document 123-109, and from Attachments 5 and 6 (no record numbers), which also are in this document.

3. Surely one purpose of a reproduction study is to generate data on the various indices that the MBIP quoted from the FIFRA guidelines. However, these same guidelines also clearly indicate that full histopathology should be done on target organs from the untreated and high-dose parents in each generation. This reflects a concern about potential chemical effects on postnatal development and provides the possibility that the results can serve as a guide for subsequent tests. Therefore, the only question is whether there is a target organ for methyl bromide. Given the neurotoxic effects of inhaled methyl bromide, which were commonly known well before the conception of this study (e.g., reviewed in records 064742 and 059183), the brain should have been designated as a target organ. CDFA also notes that the absolute weight of the brain was decreased in some parental groups in this reproduction study, thus also identifying the brain as a target organ before the study was finalized.

Having conducted the study without having a target organ studied, the MBIP's argument for why they should not be required to generate these missing data at this point is not persuasive for the following reasons.

First, before sacrifice, rats in record 058196 received only 132-145 exposures to methyl bromide versus lifetime exposures in the Dutch-sponsored study (record 059184). Still, decreased brain weights in the following 90 ppm groups were observed in record 058196: FO males, F1 males, and F1 females. Brain weights also were reduced in the F1 30 ppm females. The results were not statistically significant at the 0.05 level (Scheffe's Multiple Comparisons after a significant F-test in the ANOVA) but inspection of the data (p. D-297 of record 058196) suggests that the true p-value probably is close to 0.05.

It seems reasonable to expect that, in order to get a measurable loss in brain weight, a large amount of histological change has to occur, e.g., loss of neuronal cells. Similarly, one would expect that a histological change still could be seen as the dose is lowered to the point that the brain-weight reduction is no longer evident. Therefore, CDFA has to be concerned about these brain-weight effects in record 058196 and the best way to judge this effect is to consider the histological picture in these brains. These data should indicate whether 3 or 30 ppm is a NOAEL or a LOAEL for this brain effect in the F1 females. While the Dutch-sponsored study (record 059184) also observed reduced brain weights in its 90 ppm females sacrificed after 1 and 2 years of exposure, only the reproduction study would allow one to see if this brain effect is more pronounced when the exposures are started in utero, which is what is suggested by the greater brain weight effect seen in both F1 parents.

Second, as discussed under the heading "Oncogenicity, Rat," CDFA is seeking data to decide whether methyl bromide induced gliomas in the Dutch-sponsored inhalation study (record 059184). Various studies have shown that the rodent fetus is more susceptible than the adult rodent to the induction of brain tumors by alkylating agents (reviewed in ref. 16). It has been postulated that neurocarcinogens are able to transform some of the undifferentiated glial stem cells of the subependymal plate; as the brain develops, these cells migrate into the white matter of the brain and differentiate into the various glial cells, or in the case of the transformed cells, into (pre)neoplastic glial cells which eventually give rise to the gliomas. By this model, the induction of gliomas by transplacental exposure truly represents a perinatal effect. With the histological data for rats exposed to methyl bromide transplacentally, CDFA would have another established basis to decide whether methyl bromide deserves to be considered as a potential neurocarcinogen.

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Therefore, after considering the MBIP's comments versus what hangs in the balance because of the missing data (possibly, a NOEL in the reproduction study; an adverse effect identification in the oncogenicity study), **CDFA is requiring that the missing histological data somehow be submitted.** CDFA recognizes that this may mean that the MBIP will have to conduct a suitable supplementary study. However, this situation was entirely avoidable if the FIFRA guidelines had been followed in the first place.

Regarding the design of a supplementary study, minimally it should generate the missing brain histological data for the F1 parental rats of record 058196. A full accounting of how the brain is studied (e.g., number and locations of transversal sections) should be provided. Whether extra dosing levels are used or other endpoints (e.g., mating trials, female hormonal status) are included in the design utlimately is the MBIP's decision.

CDFA action: For the reasons discussed above, record 058196 remains an unacceptable study. Therefore, this SB 950 data requirement remains unfilled, with possible adverse effects indicated (reduced fertility, decreased pup bodyweights and some pup organ weights).

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TERATOGENICITY, RAT

MBIP comments:[5] The NIOSH-sponsored teratogenicity study using inhalation (records 026866 and 059690) is adequate for fulfilling the SB 950 data requirements for a rat teratogenicity study. Regarding the points raised by CDFA about that study, the MBIP have the following comments.

1) CDFA will be supplied the raw data for this study as soon as possible.
2) The researchers who conducted this study did not conclude, as CDFA did, that the skull ossification defect was a treatment-related effect.
3) The contribution from maternal toxicity and stress to observed developmental retardation or morphological defects in offspring remains a crucial consideration in the interpretation of teratogenicity studies. Several studies in the literature have shown that retarded ossification is a non-specific developmental effect which could be related to maternal stress (reviewed in Attachment 7). The observation of reduced skull ossification in this study may be related to maternal toxicity, rather than a specific effect on the fetus caused by methyl bromide. A decrease in bodyweight gain is considered to be an acceptable measure of maternal toxicity. Therefore, maternal toxicity was demonstrated in the high-dose group of rats because decreased bodyweight gain was evident. In fact, the initial CDFA review of this study concluded that maternal toxicity (defined by decreased maternal bodyweight) was present. In the subsequent CDFA review, there was no discussion about what additional evidence became available during the rereview which caused the reviewer to reverse the initial CDFA conclusion. From the data available, it appears that the MTD for inhaled methyl bromide during gestation is 70-90 ppm. Rabbit studies also substantiate this range since ~65 ppm is associated with adverse effects in that species.

CDFA response: CDFA has the following responses to the above comments.

1. The Battelle researchers who conducted this inhalation teratogenicity study for NIOSH did note that the slight ossification defect seen in the methyl bromide segment of their 3-chemical study was not observed in the other two segments (p. 70 of record 059690). The effect was discounted as being treatment-related because it was present in the high-dose group(s) and not in the low-dose group(s). CDFA could agree with this logic if the effect was in the low-dose group and not the high-dose group, but since there were only two treatment groups, it is entirely plausible that the effect has a NOEL at the low dose. In addition, the effect of the same magnitude was seen in both high-dose groups but not the other various groups in the methyl bromide segment of the study or in the controls for the other two segments of the study. Therefore, CDFA is persuaded to identify this skull ossification defect as a treatment-related effect.

2. Having identified the skull defect as treatment-related, the matter arises of whether this represents an effect on the fetus vs. a fetal effect precipitated by maternal toxicity. The initial CDFA review of this study by Dr. Gee designated diminished bodyweight gain as a maternal effect, with a NOEL

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[5] The MBIP's comments were abstracted from Dr. Duafala's letter (dated 11/27/89), which accompanied CDFA document 123-109, and from Attacment 7 (no record number), which is also is in this document.

of 20 ppm. However, in the rat reproduction study (record 058196), no gestational bodyweight effects were seen during either the F1a or F1b gestations in rats inhaling 90 ppm. Therefore, this reviewer (Dr. Rinkus) conducted a rereview of record 026866 in order to consider why there was a discrepancy over maternal-bodyweight effects between the rat reproduction study and the rat teratology study. In that rereview, this reviewer concluded that the evidence that the fetal effect was dependent on a maternal effect was minimal for the following reasons:

a) No mortalities and no clinical observations indicative of a toxic effect were observed in the dams.

b) The 4% bodyweight difference in the dams was really minimal and it may have owed its statistical significance to a combination of coincidental factors. First, large population sizes were used (N = 31-38 per group). Second, the study design was not a conventional, FIFRA-specified teratogenicity study. What is important about the study design that was used is that it may have resulted in an inadvertant weight bias when forming the groups before the onset of gestational treatments. Obtaining gestational groups with the same mean bodyweights does not appear to have been a criterion of the randomization process used to form these groups from the pregestational groups. This is indicated by looking at the gestational day 1 bodyweights from record 026866 (shown below) for the groups formed from the rats pregestationally exposed to 20 ppm and the groups of rats formed from the rats pregestationally exposed to 70 ppm:

	Mean Bodyweight (grams)						
	Treatment Groups[1] (ppm)						
Gestational Day	0→0	0→20	0→70	20→0	20→20	70→0	70→70
1	239	239	237	247	240	237	230*
7	269	270	266	273	268	258	257*
14	303	303	292*	303	307	295	290*
21	372	366	359	376	384	377	368

[1]Treatment groups are described in terms of their pregestational and gestational exposures, as indicated by the first and second values, respectively.
*Statistically different at the 0.05 level from the corresponding 0→0 group value (p. 27 of record 026866).

c) The delayed skull ossification was not accompanied by other instances of delayed ossification (e.g., in the sternebrae, vertebrae, or proximal phalanges in the fore paw) or by decreased fetal weight. For example, in the study by Ariyuki et al. (ref. 27) wherein dams were not fed during gestational days 14-21, all four of these sites showed delayed ossification and mean fetal weight was only 63% of the value of the control fetuses whose mothers were not fasted.

3. CDFA did not discuss previously the dam bodyweight data in terms of bodyweight gain because such data were not supplied. Using the group mean values to consider the bodyweight gain (shown below), one can see that the gestational days 1-14 bodyweight gains for the 0→70, 20→0, 70→0, and 70→70 groups are lower than the corresponding value for the 0→0 group. However, the incidence of skull defect is maximal in the 0→70 and 70→70 groups, despite the differences in the magnitude of their reductions, and no defect was seen at all in the 70→0 group. Thus, these bodyweight gain comparisons are minimally supportive of the argument that a maternal bodyweight effect (maternal stress) was the cause of the fetal skull ossification defect.

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Gestational Days	Bodyweight Gain (grams) Treatment Groups[1] (ppm) 0→0	0→20	0→70	20→0	20→20	70→0	70→70
1-7	30	31	29	26	28	21	27
1-14	64	64	55	56	67	58	60
1-21	133	127	122	129	144	140	138

[1]Treatment groups are described in terms of their pregestational and gestational exposures, as indicated by the first and second values, respectively.

4. Regarding the comparison between rabbits and rats during their gestations, because 65 ppm is toxic to rabbits, it does not follow necessarily that 65 ppm also must be toxic to rats. Assuming that being pregnant does not affect VBR and ignoring bodyweight changes during pregnancy, 7 h/d inhalation of 65 ppm methyl bromide represents doses of 13 mg/kg-d for rabbits and 26 mg/kg-d for rats. The only point really to be made by this is that the pregnant rabbit may be more sensitive than the pregnant rat.

$$\text{rabbit dose} = \frac{(252)\ [(0.893)\ (3.0/2.4)^{0.67}]\ (0.5)\ (7/24)}{(3.0)} = 13 \text{ mg/kg-d}$$

$$\text{rat dose} = \frac{(252)\ [(0.105)\ (0.250/0.113)^{0.67}]\ (0.5)\ (7/24)}{(0.250)} = 26 \text{ mg/kg-d}$$

Where: methyl bromide concentration has been converted to units of mg/m^3:
65 ppm = 252 mg/m^3, at 25°C and 760 mm Hg;
0.893 and 0.105 represent volumetric breathing rates (VBR) in units of m^3/d for a 2.4 kg rabbit and a 0.113 kg rat, respectively;
$(3.0/2.4)^{0.67}$ and $(0.250/0.113)^{0.67}$ represent scaling factors for VBRs to a 3.0 kg rabbit and a 0.25 kg rat, respectively;
0.5 is the fraction of methyl bromide absorbed from the inhaled air;
7/24 is the fraction of the day during which exposure occurs.

5. Thus, based on the data that have been submitted by the MBIP to date, CDFA feels justified in concluding that the skull defect potentially could be a treatment-induced effect in the fetuses that is not due to maternal toxicity. CDFA will reconsider this position if the individual data from the study are submitted. With the individual data, CDFA will look to see which dams had the reduced bodyweights and whether their fetuses were the ones with the skull ossification defect. Whether the skull defect is significant in the sense that it portends possible adverse reproductive human effects is not definitively known at this time. For the purposes of this stage in the SB 950 process, the adverse effect identification stage, it has been assumed that minimally delayed skull ossification is like some other common skeletal variations, e.g., retarded ossification at the other sites, supernumerary ribs, and wavy ribs. The findings of these effects in the absence of maternal toxicity is taken to indicate a potential for the test substance to affect normal fetal development in some way, which may or may not even involve the actual effect seen in the animal studies.

CDFA action: CDFA will review the supplementary data if they are submitted. In the meantime, this SB 950 data requirement remains unfilled, with a possible adverse effect (delayed skull ossification) indicated in record 026866.

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TERATOGENICITY, RABBIT

MBIP comments:[2] The MBIP has a rabbit teratogenicity study in progress at this time.

CDFA comments: None.

CDFA action: CDFA will review the completed study when it is submitted. In the meantime, this SB 950 data requirement remains unfilled. One unacceptable study (record 026865) is on file; high mortality precludes any interpretation of the results of that study.

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GENE MUTATION

MBIP comments:[2] With this submission of these publications on gene mutation, records 087801, 087802, 087808, and 087809, this data requirement is satisfied.

CDFA response: There are several points made in Attachment 1 (no record number) in CDFA document 123-109 on which CDFA would like to comment.

1. There is a questionable downplaying of the positive results in the gene-mutation assays in terms of the dose needed to elicit a positive response. It is not clear to CDFA to which "standard mutagens" is methyl bromide being compared that would justify this downplaying. In the dessicator study of Simmon et al. (record 087801), methyl bromide's mutagenic activity was detectable starting at 0.01% (100 ppm); this indicates that it is a stronger mutagen than vinyl chloride, which showed comparable mutagenic activity (without S-9) when tested at 20% in that same study. In the study by Kramers et al. (record 087802), the mouse lymphoma assays indicate that methyl bromide had detectable mutagenic activity at a concentration of 0.3 µM (30 ng/ml) and was increasingly active, without cytotoxicity, up to a concentration of 10.5 µM (1 µg/ml). By contrast, Clive et al. (ref. 28) reported the following "lowest effective concentrations" for mutagenic acitivity (TK+/-) with these direct-acting agents: EMS, 50 µg/ml; methyl iodide, 15 µg/ml; MMS, 6 µg/ml; MNNG, 5 ng/ml; β-propiolactone, 2 µg/ml; and uracil mustard, 150 ng/ml. Therefore, methyl bromide is a potent mutagen in this assay, being second in this aforementioned series of alkylating agents only to MMNG. Also, in the study by Kramers et al. (record 087802), the Drosophila testing found that prolonged exposure (6 h/d, 5 d/week, 3 weeks) to methyl bromide at 52 ppm (200 mg/m^3) was mutagenic, without being toxic. Compared to the results for what other gaseous mutagen(s) can 52 ppm be considered as a high concentration?

2. Regarding the idea that a base-substitution mutation is less important than other types of mutation because such a lesion can be repaired, CDFA is not aware of any studies that could support such an idea. If the MBIP seriously wants to argue this point, the supporting studies will need to be identified. At this point, CDFA does not believe that there exists any scientific basis for distinguishing among the various forms of genotoxicity in terms of potential genetic risk.

3. Regarding record 087799, the reporting that no DNA alkylation in the liver and kidneys was detectable in a single case of a human poisoning with methyl bromide, about which no details whatsoever were mentioned, is meaningless in its present form. However, what is clear from record 087809 which used a static inhalation system is that DNA alkylation occurred in the liver and spleen (only organs assayed) of mice that for 4 h inhaled an atmosphere that (initially) contained only 36 ppm methyl bromide.

4. In general, what CDFA concludes from these data is that methyl bromide clearly is genotoxic, which was to be expected given its alkylating ability. Therefore, CDFA must be concerned about its effects after longterm ingestion (e.g., as a residue in fumigated food commodities) and inhalation (e.g., as an occupational exposure in using it as a fumigant).

CDFA action: This SB 950 data requirement is now considered satisfied, with adverse effects (demonstrable mutagenicity in multiple assays) indicated.

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CHROMOSOME EFFECTS

MBIP comments:[2] The MBIP has submitted a Microbiological Associates Inc. protocol for a micronucleus assay in mice (CDFA document 123-108; record 085429). The route of exposure will be by intraperitoneal injection. With the submission of an acceptable micronucleus test in mice, this data requirement will be satisfied.

CDFA response: Regarding the ip route of exposure, CDFA would question why this would be used in lieu of inhalation. However, as a more fundamental question, is this proposed study really necessary, even if done by inhalation, because of the following two considerations.

1. The IARC monograph on methyl bromide (record 087806) lists methyl bromide as inducing micronuclei in mice and rats. The MBIP should try to secure the study that is the basis for this IARC designation. IARC gave as a reference an abstract, but the full reporting presumably has been published by now or otherwise is available from these researchers. Even if the MBIP conducts its own study, CDFA is requesting that this aforementioned micronucleus study in mice and rats be submitted. Also, if the results of any MBIP-sponsored testing are found not to be in basic agreement with this study, some explanation of why the results differ would need to be provided by the MBIP.

2. Micronucleus data sometimes are being generated as part of the NTP testing. Has the MBIP determined that the soon-to-be-available Brookhaven study in mice does not contain micronucleus data, chromosomal aberrations data, and (or) sister-chromatid exchange data for these exposed mice?

Regardless of the answers to the above, as requested in the previous rebuttal (5/11/89), CDFA would like to have on file the same data base in this area that is on file with EPA. This would include the sister-chromatid exchange (SCE) test mentioned in the EPA Re-registration Guidance documemt of August, 1986, if this still is going to be provided to EPA.

CDFA action: CDFA will review the chromosomal data when they are submitted. In the meantime, this SB 950 data requirement remains unfilled. Records 035750 and 087806 indicate a possible adverse effect (induction of SCEs and micronuclei).

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DNA DAMAGE

MBIP comments:[2] The MBIP has submitted a Microbiological Associates Inc. protocol for the detection of single-strand breaks (SSB) in rat testicular DNA by the alkaline elution assay (CDFA document 123-108; record 085428). The route of exposure will be by inhalation. With the submission and acceptance of this alkaline elution study, this data requirement will be satisfied.

CDFA response: It is the general practice of CDFA not to analyze protocols as it does data submissions because it simply does not have the personnel to commit to reviewing protocols in the timely fashion that would be needed typically in these situations. However, in this case since the procedure is without FIFRA or TSCA guidelines, the protocol has been reviewed briefly. Before making some comments, it should be understood that justifiable, defensible science is always the guidance in any toxicological assay. Also, even when there are no guidelines *per se*, the testing should be in general agreement with the common strategies that are used in the FIFRA and TSCA guidelines for toxicological testing, in this case, for selecting the highest dose tested and for conducting and reporting the inhalation exposure. If deviations from the guidelines, or in this case from the original procedure (ref. 29), are necessitated by circumstances, CDFA requests that the circumstances be explained adequately, with presentation of data when appropriate.

Regarding the protocol, CDFA would make the following four comments.

1. A least two CDFA scientists have hands-on experience with the DNA alkaline elution procedure using mammalian cells in culture. Their experience with the assay leads them to request that the actual elution curves be submitted in order to facilitate the review of the study when it is submitted.

2. The cover letter by Dr. Duafala in CDFA document 123-109 (dated 11/27/89) identifies this assay as a spermatozoal alkaline elution assay. CDFA presumes that this is an oversight since this assay is performed with a mixture of cells isolated from the testes (spermatogonial cells, spermatocytes, Serotoli cells--but not spermatozoa) and that there is not some confusion between the method of Skare and Schrotel (ref. 29) with the method of Sega et al. (ref. 30). The latter also measures DNA SSB but in spermatozoa taken from the cauda epididymis; the latter also has been used to test ethylene oxide (ref. 31), the positive control in the Microbiological Associates protocol.

3. The protocol (p. 2 of record 085428) indicates that the rats will be $\geq$ 8 weeks old. Given that this assay addresses the induction of SSB in the DNA of gametic tissue, the rats when exposed to methyl bromide should be at their minimum breeding age or at least sexually mature histologically speaking (full spermatogensis should be evident in the testes). This would place the minimum age of the rats at $\sim$14 weeks.

4. The protocol indicates that the 15th hour of the elution will be used to derive the elution rate (p. 8 of record 085428). This is only appropriate when the elution curve is decidely linear. Otherwise, it would be preferable to use the initial time points to determine the elution rate, e.g., using linear regression to derive the slope (elution rate).

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Finally, as requested in the previous rebuttal (5/11/89), CDFA would like to have on file the same data base in this area that is on file with EPA. This would include the unscheduled DNA synthesis test using rat hepatocytes mentioned in the EPA Re-registration Guidance document of August, 1986, if this still is going to be provided to EPA.

CDFA action: CDFA will review the completed alkaline elution study when it is submitted. Technically, this SB 950 data requirement was satisfied by record 903095, with an adverse effect (increased mitotic recombination) indicated.

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NEUROTOXICITY

Note: Under SB 950, this category generally concerns organophosphate-induced delayed neuropathy. CDFA's concern about the neurotoxic potential of methyl bromide is discussed under the heading "Regarding the Assessment of the Neurotoxic Potential of Methyl Bromide."

NOTE:

1. All studies received by the Medical Toxicology Branch up to 6/4/90 have been considered in this response.

2. Pursuant to his request, this rebuttal (R900604) and the latest SUMMARY OF TOXICOLOGICAL DATA (T900604) will be sent also to William Burnam, Deputy Director, Health Effects Division (H7509C), Office of Pesticide Programs, US EPA, 401 M Street SW, Washington, D.C., 20460.

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APPENDIX TO ATTACHMENT 1

Table 1. "Decay" Parameters and Predicted Postfumigation Residues for the Loss of Organic Methyl Bromide from Fumigated Food Commodities.

Food Commodity (Reference)	Postfumigation Storage Temperature	κ	Half Life (h)	Predicted Concentrations (mg/kg) at postfumigation times[1] 1 week	2 weeks	3 weeks	4 weeks
grapefruit (1)	24°C	.0847	8.2	7×10^{-6}	4×10^{-12}	3×10^{-18}	2×10^{-24}
peach (2)	2.5°C	.2581	2.7	1×10^{-18}	2×10^{-37}	3×10^{-56}	5×10^{-75}
nectarine (2)	2.5°C	.2255	3.1	4×10^{-16}	1×10^{-32}	4×10^{-49}	2×10^{-65}
cherry (2)	2.5°C	.1355	5.1	1×10^{-9}	2×10^{-19}	2×10^{-29}	3×10^{-39}
pear (2)	2.5°C	.0833	8.3	8×10^{-6}	7×10^{-12}	6×10^{-18}	5×10^{-24}
plum (2)	2.5°C	.0502	13.8	.002	5×10^{-7}	1×10^{-10}	2×10^{-14}
pistachio nuts (3)	15.5°C[2]	.0148	46.8	.832	.069	.006	5×10^{-4}
	26.6°C[2]	.0132	52.5	1.089	.119	.013	.001
rice (6)	Not Stated	.1811	3.8	6×10^{-13}	4×10^{-26}	2×10^{-39}	1×10^{-52}
white maize (6)	Not Stated	.0477	14.5	.003	1×10^{-6}	4×10^{-10}	1×10^{-13}
oats (6)	Not Stated	.0470	14.8	.004	1×10^{-6}	5×10^{-10}	2×10^{-13}
wheat (6)	Not Stated	.0277	25.0	.095	9×10^{-4}	9×10^{-6}	8×10^{-8}
cacoa beans (6)	Not Stated	.0165	41.9	.623	.039	.002	2×10^{-4}

[1]Based on the equation: $C = C_0 e^{-\kappa t}$

where C = the concentration of methyl bromide in mg/kg as a function of postfumigation time

C_0 = the concentration of methyl bromide immediately after fumigating; for calculation of residue levels at 168 to 672 h, a value of 10 mg/kg was used arbitrarily for the sake of comparison

κ = the "decay" constant calculated by CDFA from the data in the cited reference or provided by the authors in the case of ref. 1 (i.e., the reciporical of -11.8 is -0.0847)

t = postfumigation time in hours.

Half-life = (ln 0.5) ÷ κ

[2]The report does not clearly state the postfumigation temperature; these temperatures are inferred from concurrently run studies with insect eggs.

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APPENDIX 1 (continued)

Table 2. Times When First Stomach Tumor Was Detected in Rats Treated with Some Known Stomach Carcinogens.

Stomach Carcinogen (Reference)	Dose (mg/kg)	Method of Exposure	Vehicle	Dosing Regimen	Time after Start of Exposure(s) That 1st Tumor was Detected[1]
MNU[2]	40	gavage	saline	only once	week 45
(10)	40	gavage	saline	1 d/w for 3 w	week 9
MNNG[3]	~7[4]	drinking water	water	7 d/w for 31 w	week 23
(11)				7 d/w for 15 w	week 35
β-propiolactone (12)	30	gavage	salad oil[5]	2 d/w for 50 w	week 32
ethylene oxide (12)	30	gavage	salad oil	2 d/w for 107 w	week 79
propylene oxide (12)	60	gavage	salad oil	2 d/w for 109 w	week 79
epichlorohydrin (13)	10	gavage	water	5 d/w for 104 w	week 95
methyl bromide (8)	50	gavage	peanut oil	5 d/w for 25 w	week 25

[1]These times refer to the detection of squamous-cell carcinoma of the forestomach, except for adenocarcinoma of the glandular stomach in the case of the MNNG entries.
[2]N-methyl-N-nitrosourea, MNU.
[3]N-methyl-N'-nitro-N-nitrosoguanidine, MNNG.
[4]Estimated by assuming a daily water intake of 25 ml/rat and a bodyweight of 0.3 kg for rats allowed to drink ad libitum water containing 80 mg MNNG per liter.
[5]Given as a suspension in salad oil.

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APPENDIX 1 (continued)

Table 3. Some Literature Values for Gliomas in Untreated Wistar Rats.

Conducting Laboratory[1]	Wistar Sub-Strain	Duration of Study	Sex	# Gliomas / # Examined	Age (days) When Diagnosed	Ref.
TNO-CIVO	Cpb: WU	2.3 y	M	2/70	Not Stated	22
			F	0/69		
TNO-CIVO	Cpb: WU	2.0 y	M	0/50	Not Stated	23
			F	0/50		
CSLA-AUW	RIV-TOX-(M)	2.7 y	M	2/247[2]	Not Stated	24
			F	0/249[2]		
NIPH	SPF-TOX[3]	2.5 y	M	3/192	920, 920, 890	25
			F	0/192		
	Cpb (pre-1970)[4]	2-2.5 y	M	3/197	650, 682, 730	
			F	0/182		
	Conv (pre-1970)[4]	"long-term"	M	1/83	667	
			F	0/83		

[1]The conducting laboratories were: TNO-CIVO, TNO-CIVO Toxicology and Nutrition Institute (Zeist, The Netherlands); CSLA-AUW, the Centre for Small Laboratory Animals of the Agricultural University of Wageningen (Wageningen, The Netherlands); the National Institute of Public Health (Bilthoven, the Netherlands).

[2]The results of 5 groups receiving different animal and human diets in this study were combined; range for individual groups: 0/50 to 1/48.

[3]This may be the same strain as RIV-TOX-(M) since reference 24 cites reference 25 as a justification for using this strain in the study.

[4]These data were from studies conducted before 1970 at this laboratory; these data are for animals that served as controls; "long-term" was not defined in the case of the data for the conventional Wistar rat stock (Conv).

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